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IS COPPER ESSENTIAL FOR IRON UTILIZATION?

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It is now generally accepted that copper plays a physiological rôle in the utilization of iron for the formation of hemoglobin. The literature has been reviewed by Elvehjem (1) and by Smith (2). Cunningham (3) pointed out that rats on a milk diet may die soon after the milk diet is commenced and show low hemoglobin; others die after a longer period of milk feeding and without marked anemia, and others live for still longer periods without exhibiting dangerous lowering of hemoglobin. The benefit to rats restricted to milk resulting from copper administration in many cases has been fully demonstrated. The rôle of copper deficiency in the anemia in such animals is not satisfactorily explained. The high mortality of rats restricted to fluid milk, certainly involves some factor other than anemia. Because of the high water content of the food, underfeeding occurs and frequently results in inanition. Such rats suffer from cecumitis, stasis and infection, particularly pneumonia.

In 1931-32 the writer reared three generations of rats, in glass cages, on a modified powdered milk diet containing less copper (0.82 mgm. per kgm. of dry food) than is usually found in uncontaminated fresh milk. It was believed that the reduction of the water content of the milk diet changed entirely the outcome of the experiment. The study here reported represents an extension and elaboration of that experiment.

An evaporated milk was prepared from Holstein and Guernsey milk, collected in glass bottles. This was allowed to stand overnight at 40°F. The lower half was siphoned into a pyrex distilling flask and evaporated, at 20 to 30 mm. pressure, to half its volume. The evaporated milk was mixed with the top half and cream. Analysis showed that the copper content had not changed. The following experiments were made with the specially prepared milk to test the possibility that reduction in the water content of the food would make possible the consumption of sufficient food

for normal growth and hemoglobin formation. As controls, rats were fed the specially collected fluid milk exceptionally low in copper and another group fluid milk to which potassium-sodium tartrate was added for its diuretic property.

The rats used were from a pied colony fed the Steenbock stock ration. They were kept in galvanized wire cages with glass feeding cups. They were on half-inch wire screens which prevented access to feces. When the young were 10 days old, a mother with her young was placed in the cage and was fed nothing but evaporated milk supplemented with ferric chloride prepared from pure iron wire (copper-free), and 0.7 gram of yeast daily. Since the yeast contained some copper it was placed beyond the reach of the young. When the young had reached weights of 40 to 45 grams, twenty-four were selected and placed on a diet of whole fluid milk until they had developed severe anemia. Their hemoglobin (Newcomer) was reduced to 2 to 3.5 grams per 100 ml. of blood in three weeks. These rats were then divided into six groups of four animals. Each rat was kept in a separate cage. Group 1 was given the evaporated milk, specially prepared. Group 2 received the evaporated milk plus ferric chloride equivalent to 0.25 mgm. of iron per day. Group 3 received fluid milk plus 0.25 mgm. of iron as chloride and copper sulfate equivalent to 0.01 mgm. of copper per day. Group 4 received fluid milk plus 0.25 mgm. of iron as chloride. Group 5 received fluid whole milk plus 0.25 mgm. of iron and 0.2 mgm. of the diuretic theobromine daily. The rats refused the milk containing the theobromine, so sodium potassium tartrate of C. P. grade which gave no test for copper was substituted. Group 6 was left on fluid milk alone.

In less than a week after the division into groups the rats given no supplement, other than iron (groups 1, 2, 4 and 6) began to die. The stomachs were distended and the ceca were congested, while the remainder of the alimentary tract was empty. With the death of the first two animals it was noticed that all the living animals not receiving copper or the tartrate were suffering from various degrees of constipation. The majority of them were sick. The tartrate relieved the condition; so did mineral oil, copper, and an alcoholic extract of yeast. However, if the rats were already ill, nothing seemed to save them. Twelve rats from this series were saved.

When the rats were three months of age, they were placed on a diet of evaporated milk, purified iron and sucrose shown to be free from copper. The sucrose was added in the proportion of 50 grams to a liter of whole milk. The rats were paired at this time. Groups 4 and 6 were kept on a diet of whole milk and iron but did not gain weight as rapidly as the others. Their growth curve flattened at 160 grams. When handled, they seemed to be all skin and bones. At four months they, too, were placed on the

modified evaporated milk diet. The feeding of copper was continued with group 3, but they did no better than those without after the constipation was cured. The growth curves for all the rats during the fourth and fifth months flattened out and, at the end of the experiment, the females were about 30 grams under weight. The hemoglobin was somewhat low, 10.5 to 12 grams per 100 ml. No pregnancies occurred even though some of the rats had been paired with stock rats.

Copper in this series appeared to counteract constipation caused by an exclusive milk diet. It stimulated the appetite. The final weights and hemoglobins were no greater in the copper fed animals than in those that did not receive copper.

TABLE 1

Summary of series I

	GROUP					
	1	2	3	4	5	6
Original number of rats	4	4	4	4	4	4
Number given a supplement	3	4	4	4	4	2
Supplement given:						
Yeast extract	1	1		1		
Copper	1*	1*	4	1		1*
Mineral oil	1*	1		1*		
Tartrate		1*		1*	4	1
Number saved	1	3	3	2	2	1

* Indicate the rats that died. The rat from group 3 died with an ulcer at the apex of the heart. The two from group 5 died with severe diarrhea, evidently, from too much tartrate. The supplement, in each of the other cases where death occurred, was not given soon enough. Two of the rats died of pneumonia.

Series II. This set of experiments was started in February. The rats were weaned on March 19. The object was to obtain further information concerning the action of copper as an irritant and stimulant to the gastro-intestinal tract.

Nineteen albino rats were kept in individual cages on a diet of fluid whole milk. Again anemia developed in two to three weeks. This time one third of the rats were given copper and iron, one third copper, manganese and iron, and the other third iron alone as a supplement to the fluid milk. All of the rats developed diarrhea (either an epidemic or from physic given one of the cows furnishing the milk). The milk was boiled and some of the cream removed, and one drop of 10 per cent halibut liver oil (plain) in olive oil was given every day as long as the cream was removed. Nine of the rats died. All six of the rats on fluid milk supplemented with iron alone lived. At three months of age they were placed on the evaporated

milk-sucrose-iron diet. At five months of age the females had an average weight of 261 grams and the males 300 grams and a hemoglobin value of 14.0 to 15.0. Three litters of young had been dropped and weaned.

Series III. This series was started about the first of May, when the cows were on pasture. The rats were weaned on May 28. There were 29 young available for this series. Ten rats were fed whole milk supplemented with iron, and 19 evaporated milk supplemented with iron, while four, two from each group, were given a further supplement of copper. Seven of the rats (four of which were the copper controls) died of causes other than anemia. Twenty-two of twenty-five receiving whole or evaporated milk and purified iron lived, gradually recovered from anemia and were in good health at the end. The males weighed an average of 180 grams and the females 143 grams at 3 months of age.

CONCLUSIONS

It appears from the data presented that: 1, copper is not an essential element in nutrition; 2, the excessive water content of fluid milk and its incorrect balance of protein, fat and carbohydrates makes it impossible for the animals to eat sufficient food for growth and hemoglobin formation; 3, the rôle of copper is to facilitate intestinal elimination.

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HAIR GROWTH IN ADRENALECTOMIZED, AND ADRENALECTOMIZED THYROXIN-TREATED RATS

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Numerous papers dealing with the survival and activity of adrenalectomized rats have appeared recently. No attention, however, has been given to the effect of adrenalectomy on the hair coat in this animal despite the fact that hair growth is frequently affected in humans by alterations in the cortical portion of the adrenal.

In rabbits (1) partial removal of the adrenal cortex results in increased heat production, and in mice (2) total metabolism increases upon extirpation of the adrenals, but no rise is noted in either upon removal of both the adrenals and the thyroid. These results suggest that the activity of the thyroid is inhibited in some way by the adrenal cortex. Thyroxin administration may possibly be more effective on tissue growth in the adrenalectomized animal than in the normal rat.

The present investigation has, therefore, been undertaken to learn 1, what effect adrenalectomy has on the second coat of hair which appears externally on the dorsum of the white rat about the 38th day (3); 2, if a greater acceleration of hair and tissue growth can be induced by adrenalectomy and thyroxin administration than from thyroxin with the adrenals intact. Experiments on the latter problem may give some idea as to how and to what extent the adrenal cortex regulates the thyroid.

METHODS. The number of rats in the litter (Wistar Institute strain) was reduced to six or seven individuals soon after birth so that a better growth and uniformity (a weight of 40 grams or more serving as a basis) would exist at the beginning of the experiments on the 22nd day. The hair was then removed from a considerable area of the back, and with the rat under ether anesthesia and using the dorsal approach, the adrenals with their capsules were excised. Four animals of the litter were adrenalectomized in this manner while the controls were subjected to a mock adrenalectomy. Adrenals were also removed in some rats via the ventral approach so that any effect on the hair growth could not be due to injury and increased vascularization of the dorsum, but only to the absence of the adrenals. Besides the regular ration (Purina dog chow and water), 2.5 per cent salt solution was kept accessible. As previously described

(4), the backs of the rats remained nude until the hair made its second growth, the time of which was carefully ascertained in these experiments to determine the extent of precociousness and the rate of growth.

The thyroxin experiments were usually begun on the 21st day, and the litter was divided so that two individuals received thyroxin, two were adrenalectomized, and two were adrenalectomized and given thyroxin. Different amounts of the hormone were administered subcutaneously daily just before or following adrenalectomy. Such variations enabled one to determine the amount necessary for a maximum effect, the amount that the animal would tolerate, and the best time for administration.

TABLE 1

The effect of bilateral adrenalectomy on the time of appearance of the second growth of hair in the albino rat

NUMBER IN LITTER	NUMBER ADRENALECTOMIZED	AGE WHEN ADRENALECTOMIZED	AGE OF ADRENALECTOMIZED WHEN THE HAIR APPEARED	AGE OF CONTROL WHEN HAIR APPEARED
		days		
6	3	23	32, 32, 32	36, 36, 36
4	2	23	32, 32	37, 38
7	3	23	33, 33, 34	39, 40, 40, 41
7	5	24	35, 35, 36, 37, 38	41, 41
6	4	25	35, 35, 35, 35	37, 40
6	4	22	32, 32, 33, 33	38, 40
4	2	23	33, 34	37, 39
6	4	22	35, 35, 35, 35	40, 41
6	4	22	33, 34, 33, 32	37, 37
6	4	22	33, 33, 34, 34	37, 37
Average day when the hair appeared in the control				38.4
Average day when hair appeared in adrenalectomized rats				33.8
Average number of days of precociousness				4.6

RESULTS. *The effect of adrenalectomy.* None of the 35 adrenalectomized animals (table 1) died before the 33rd day, and the nine dying before the 45th day lived on the average of 16 days after adrenalectomy. The salt solution was removed from the cages on the 45th day and five died soon thereafter. The remaining 21 survived for only a short time. Between the 23rd and usually the 38th days, the average gain in weight of the 23 controls was 42.9 grams, while the gain of the 35 adrenalectomized rats over the same period was 27.7 grams.

The adrenalectomized rats produced hair externally three to eight days (average 4.6 days) before the litter mate controls as may be seen in the table. Four days might not seem much of an acceleration in the rate of growth, but, when the rest interval of the hair follicle is only 16 to 17 days (3), the extent of precocity is at once recognizable. The hair

was better in quality and the back was more evenly covered in the adrenalectomized animal, despite the fact that they neither gained as much in weight nor were they as healthy as the control litter mates. Even the best growth frequently occurred in the poorest experimental rats, and some of these died soon after the growth was well started. Thus, a precocious growth had occurred in animals showing the symptoms of chronic adrenal insufficiency.

To determine whether this precocity of hair growth was due to the lack of the cortical or the medullary portions of the adrenal, the adrenals of 13 animals were removed, cut, and implanted into an incision in the kidney.

As may be seen in table 2, one animal produced hair precociously on the 34th day. When sacrificed on the 42nd day, histological examination

TABLE 2

The effect on the time of appearance of the second growth of hair when the adrenals are removed and implanted into the kidney

NUMBER IN LITTER	NUMBER ADRENALECTOMIZED AND IMPLANTED	AGE WHEN OPERATED	AGE OF OPERATED WHEN HAIR APPEARED	AGE OF CONTROL WHEN HAIR APPEARED
		days		
5	3	24	39, 39, 40	40, 42
5	3	21	39, 39, 38	38, 39
5	3	23	34*, 39, 40	37, 40
6	4	22	39, 37, 37, 40	38, 40
Average day when the hair appeared in the control				39.3
Average day when the hair appeared in operated				38.4
Average increase in weight of controls (23–36 days)				34.7 grams
Average increase in weight of operated (23–36 days)				30.9 grams

* No cortical material found.

revealed neither accessory tissue nor cortical implant. In three other instances a very small area of hair appeared over one kidney on the 35th day, and the rest of the back remained nude until the 38th and 39th days. The fact that only small amounts of cortical tissue were found in these rats on histological examination indicated that there was not enough cortical material at first to suppress directly or indirectly hair growth, but as the cortical material recovered and hypertrophied, hair growth was retarded. Associated with the normal time of appearance of the hair in the remaining nine rats, as compared to litter mate controls, was much cortical tissue. The medullary material had completely degenerated while the cortex with its normal appearing cells and demonstrable zones gave every indication of normal functioning. The twelve rats, in which cortical material persisted, gained almost as much in weight

and appeared just as healthy as the controls. Thus, where cortical material existed, the hair growth was normal, and it appeared that the lack of medullary material was without effect.

Cortical extracts were then used in the attempt to replace the effects caused by adrenalectomy. Daily subcutaneous injections of 0.1 to 0.2 cc. of Eschatin or a similar amount of Wilson's adrenal cortex extract from the 21st day until the hair grew in adrenalectomized animals failed to prevent the earlier growth of hair. Charcoal hormone adsorbate (kindly supplied by Merck and Company) was fed in Purina fox chow so that the daily consumption per adrenalectomized individual from the 21st to the 34th day was 1.2 units. The extract was ineffective as the hair grew precociously. Less growth, less activity, and the general condition of the rats indicated that this hormone had not prevented symptoms of adrenal insufficiency.

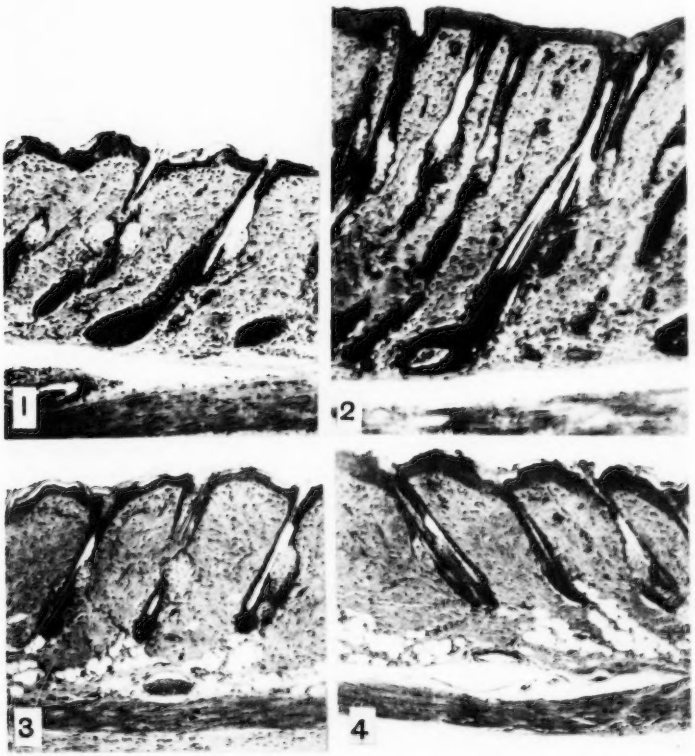
Whether larger amounts of cortical extracts will replace the effects of adrenalectomy has not been determined. The results were disappointing yet not surprising since Richter (5) was unsuccessful in establishing normal activity after adrenalectomy with similar hormones.

The effect of adrenalectomy and thyroxin. Adrenalectomized animals could usually tolerate only 0.1 mgm. thyroxin (Squibb) daily for three successive days following the operation. Few survived four injections, and some even succumbed to three administrations about the 26th or 27th days. Litter mates, which had either received a similar amount of thyroxin or had only been adrenalectomized, were killed upon the death of the adrenalectomized, thyroxin-treated animal. Areas of skin from the dorsum of the various animals were fixed in Bouin's fluid. After several hours of fixation, thin sections were cut free-hand from each specimen and the condition of the hair follicle could usually be determined under the dissecting scope. If doubt existed concerning the stage of the follicle, pieces were imbedded after the usual infiltrating procedure, sectioned, and stained in hematoxylin and eosin.

In those animals dying from adrenalectomy, thyroxin-treatment on the 27th day, the buds for the second coat of hair showed a two-days' growth (fig. 1). The hair follicles of the adrenalectomized animals, on the other hand, and those only thyroxin-treated were still in the resting condition (figs. 3 and 4). It was, therefore, very evident that the thyroxin in the adrenalectomized rats had stimulated growth to begin on the 24th to 25th days, or three to four days after the first injection.

Several of the adrenalectomized, thyroxin-treated animals survived and their hair appeared externally on the 29th and 30th days. Figure 2 shows a follicle of a 29-day-old individual, and the second hair in its growth has almost reached the surface of the body. Animals just adrenalectomized produced hair externally on the average of the 33rd day, while

the hair on those receiving only thyroxin appeared on the 36th and 37th days. The hair grew on the untreated controls (the seventh individual in a few litters) on the 37th day. Thus, the presence of the adrenal in those given thyroxin had apparently prevented the effects of the hormone.



Figs. 1 and 2. Hair growth in 27 and 29-day-old rats, adrenalectomized on the 21st day and thyroxin-treated on the 21st, 22nd, and 23rd days.

Fig. 3. Resting stage in a 27-day-old rat, adrenalectomized on the 21st day.

Fig. 4. Resting stage in a 27-day-old rat, given 0.1 mgm. of thyroxin on the 21st, 22nd, and 23rd days.

Two thyroxin injections of 0.1 mgm. each following adrenalectomy failed in most instances to induce the precocious growth. Administration of thyroxin on the four days previous to adrenalectomy was usually not very effective. Failure also occurred frequently when three 0.05 mgm. doses were used. Thyroxin in 0.1 mgm. subcutaneous injections on three

successive days following adrenalectomy, therefore, provided the best results. There was, however, individual variation and a delicate balance between the amount effective and the amount tolerated.

The effect of thyroxin on normal animals. Since the adrenal had prevented the effects of three or four daily injections of thyroxin into normal animals, 0.1 mgm. of thyroxin was administered daily from the 21st to 34th days to determine whether or not successive doses for longer periods would overcome the regulatory and inhibitory influence of the adrenal cortex. The eight animals treated in this way gained in weight on the average of 42 grams, while the average increase of four controls was 54 grams. Hair in the thyroxin-treated appeared externally sometimes a day sooner. On the 38th day the average weights of the adrenals in both the eight experimental animals and the control animals were practically the same. Therefore, there had been no hypertrophy in the weight of the adrenals to overcome the effects of the thyroxin. Larger doses of 0.2 mgm. daily from the 18th to 26th days, likewise produced no acceleration of hair growth, but when the amount was increased to 0.4 mgm. daily for four days beginning on the 18th day, the hair appeared precociously on the 30th day.

Pituitary administration in adrenalectomized animals including rat implants from the 21st to 28th days, 0.1 cc. Antuitrin (Parke, Davis & Co.) daily from the 21st to 29th days, or 0.1 to 0.5 cc. of Antuitrin growth hormone (Parke, Davis & Co.) for the six days following adrenalectomy has failed to produce a greater acceleration of hair growth than does adrenalectomy alone. Pituitary administration so far has also not induced any precocity in normal rats when given from the 18th to 34th days.

Discussion. The second growth of hair in the normal immature rat appears externally about the 38th day on the dorsum, and when the rats are bilaterally adrenalectomized on the 22nd day, the hair grows about four days sooner.

Evidence from two sources indicates that the lack of the cortical portion is responsible for this precocity. In the first place, animals die from adrenal insufficiency after the hair growth is well started, indicating that the precociousness is occurring in the absence of cortical tissue. Secondly, implants of the adrenal into the kidney show that the precocity is not due to the lack of the medullary substance since the growth is normal when the cortical portion survives and the medullary tissue degenerates. By creating comparable injuries in experimental and control animals and excising the adrenals via the ventral approach in others, the acceleration cannot be assigned to injury and increased vascularization.

The fact that a precocious growth in the rat resulted from the lack of

cortical material seems exactly opposed to our general conception, for, in the human, hirsutism and precocious sexual maturity have frequently been associated with hypertrophy of the adrenal cortex. It is, however, not known that this increased hairiness is due to excessive production of normal constituents. Kendall (6) suggests that the symptoms seen in tumors of the adrenal cortex may even be due to production of an abnormal secretion. Recent experiments by Fitzhugh (7) indicate that cortical hormone produces hypertrophy of the ovary, but others (8) (9) find that excessive quantities produce no effects on the reproductive system in normal immature rats.

The earlier appearance of the hair upon adrenalectomy is in accord with the results of other investigators as increased heat production occurs in partially adrenalectomized rabbits (1), and in mice (2) total metabolism increases after adrenalectomy.

Administration of 0.1 mgm. of thyroxin on the 21st, 22nd, and 23rd days following adrenalectomy induces hair growth externally on the 29th and 30th days, or from three to four days sooner than does adrenalectomy alone. A much earlier and greater administration of the thyroxin (0.4 mgm. daily, 18th to 22nd days) is necessary to produce a similar effect in the normal animal, indicating that the adrenal does inhibit the effectiveness of thyroxin.

Since small amounts of thyroxin produce such a precocity in the adrenalectomized animal, it seems to indicate that there is not much available thyroxin normally in the animal at the time when the injections are made. Possibly the thyroid becomes more active at intervals, and its hormone is effective at once in the adrenalectomized animal, while in the normal rat there is not any acceleration because of the inhibitory influence of the adrenal. My experiments do not show that there is necessarily an earlier activity of the thyroid upon adrenalectomy.

SUMMARY

It has been found that adrenalectomy results in an earlier growth of the hair in the rat, and that the lack of the cortical portion is responsible for this precocity.

Administration of three 0.1 mgm. doses of thyroxin subcutaneously to adrenalectomized rats induces hair growth sooner than does adrenalectomy. Earlier administration of much larger amounts of the hormone is necessary to produce a similar precocity in unoperated rats.

From these experiments it is, therefore, evident that hair growth can be accelerated in the rat by adrenalectomy and by thyroxin treatment, and that the adrenals inhibit the effectiveness of thyroxin.

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BLOOD SUGAR RECOVERY FROM INSULIN HYPOGLYCEMIA AFTER SECTION OF THE SPLANCHNIC NERVES[1]

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In a recent paper (1) it was shown that bilateral extirpation of the adrenal medulla does not prevent recovery from insulin hypoglycemia. The mechanism of restitution of blood sugar to normal values can be clearly distinguished from the emergency action of the adrenal medulla at emergency blood sugar levels. The present paper deals with the extent to which the sympathetic nervous system, or parts of it, may be held responsible for recovery from hypoglycemia. The data here presented verify the nervous control of the emergency mechanism and show what happens to the blood sugar with various inactivating procedures applied to portions of the nervous system concerned.

Well nourished, healthy male cats weighing 3.5 to 4 kgm. were used. Preliminary to the experiments the animals were kept under observation for one to two weeks to determine whether latent upper respiratory infections existed. Food was removed from the cages 18 hours before the experiments were performed. Blood for analysis was obtained from the femoral vein at the intervals indicated and sugar determinations were made on tungstic acid filtrates by the Shaffer Hartman method. Two units of insulin (Lilly) per kilogram contained in a volume of 1 cc. were injected into the femoral vein. Blood sugars were recorded as per cent change from the initial value. The curves represent mean values and the number of observations are recorded.

In performing the nerve operations we were guided by Stiemen's (2) excellent description of the sympathetic system in the cat.

In the cat the adrenal glands receive nerve fibers from the thirteenth dorsal and first, second and third lumbar sympathetic ganglia. The latter are connected by rami communicantes with the twelfth, thirteenth, fourteenth, fifteenth and sixteenth spinal nerves. In the following report the splanchnic nerves are considered to be fibers which traverse the

[1] Grateful acknowledgment is made to Eli Lilly & Co. for a grant in aid and for liberal supplies of insulin.

thirteenth dorsal and first lumbar sympathetic ganglia and receive rami communicantes from the twelfth, thirteenth and fourteenth spinal nerves.

All operations were performed under nembutal anesthesia and with strict asepsis. The nerves were exposed through an upper mid-abdominal incision and the following procedures were carried out, 2 cats being used for each type of experiment: *a*, bilateral division and resection of 2 cm. of major and minor splanchnic nerves just after emergence from the diaphragm; *b*, bilateral removal of the lumbar sympathetic chain from just below the first to just above the fourth ganglion; *c*, *a* and *b* combined; *d*, *a* and *b* plus removal of the celiac ganglion; *e*, *d* plus wide excision of the celiac plexus. Observations on the response to insulin were made 3 weeks to 6 months after the operations. Careful dissection at autopsy showed that the operations were performed satisfactorily. Gross and microscopic examination of the adrenal glands showed no abnormalities. Periods of one week at least but usually more were allowed to elapse between experiments. Animals with denervated adrenals remain in a weakened condition for a variable time after the injection of insulin.

Results. All the data are shown in the form of curves in figure 1.

Controls. Five normal cats after injection of 2 units of insulin per kilogram showed a blood sugar course as described previously (1). Hypoglycemic symptoms did not appear and complete recovery occurred within 300 to 360 minutes.

Lumbar ganglionectomy: In 4 experiments in cats with upper lumbar ganglionectomy (group *b*) the results were essentially the same as those observed in normal cats.

Splanchnic section: In animals with divided splanchnic nerves (groups *a*, *c*, *d* and *e*—10 experiments) the results were very much the same as with medullectomized animals (1). Percentage decrease in blood sugar is shown in figure 1 and milligrams of blood sugar per 100 cc. in table 1. During the time of low blood sugar values all of the animals were in severe hypoglycemic shock and often appeared to be in extremis. In 8 experiments repeated violent convulsions developed; the incidence of convulsions is indicated in figure 1. In every instance recovery from symptoms occurred and the blood sugar returned to the initial level within 360 to 420 minutes.

Two unsuccessful experiments (exitus in hypoglycemia) emphasize the importance of physical condition. One cat had not completely recovered from the strain of a previous insulin experiment (5 day interval). The other was not recovering from an attack of "sniffles." Autopsy showed patchy consolidation of lungs.

All the experiments in which the splanchnic nerves were sectioned are reported together because there were probably no significant differences in the blood sugar curves in the various sub-groups. Such distinctions

as well as distinctions between the curves for denervation and medullectomy previously reported would require larger numbers of experiments.

Discussion. The experiments here presented together with those previously recorded show that two procedures, section of the splanchnics and the removal of the adrenal medulla, abolish the sympathico-adrenal emergency mechanism but do not abolish the blood sugar recovery mechanism. Neither epinephrin secreted by the adrenal nor nerve impulses carried by the splanchnic nerves can be held responsible for the rise of blood sugar from hypoglycemic to normal levels. Furthermore it is shown that bilateral section of the splanchnics in cats is sufficient to completely abolish the protection against insulin shock afforded by the normal sympathico-adrenal activity. This location of the responsible nerve paths—

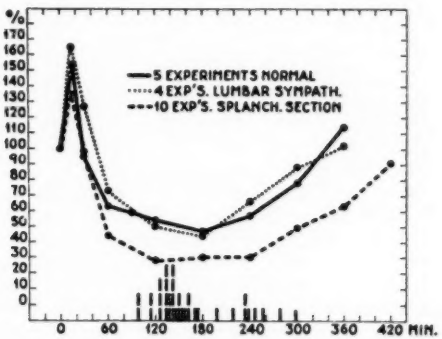

Fig. 1. The effect of 2 u/kgm. of insulin given intravenously. The curves represent mean values. Each individual initial value was taken as 100 per cent. The data were obtained from normal cats, cats with section of the splanchnic nerves and cats with lumbar sympathectomy as described in the text. The blocks at bottom of chart indicate the time and frequency of convulsions in the ten experiments with splanchnic section.

within the splanchnic nerves rather than in association with lumbar ganglia—agrees with that found by Barcroft (3) for spleen contraction.

Observing the effects of section of the splanchnic nerves and local anesthesia in the splanchnic region of dogs Rupp (4) showed that blood sugar recovery after insulin took place in every case although he recorded considerable retardation. Britton, Geiling and Calvery (5) reported 38 experiments in 9 cats with moderate doses of insulin after adrenal inactivation. In all of the experiments hypoglycemic symptoms developed and in 24 of these convulsions occurred. They state: "These animals furthermore would apparently have succumbed had no steps been taken to promote their recovery." We have so far carried out 49 experiments on adrenal inactivation in which 47 recovered, 40 of these showing definite

hypoglycemic symptoms. Of this number 23 showed one or more convulsions. (Sixteen experiments in 5 medullectomized cats (1), 21 experiments in 4 medullectomized dogs (1), 12 experiments in 8 cats with splanchnic sections.) Reasons assigned for the failures are stated above.

Our observations are not at variance with those of Dworkin (6) on the failure of completely sympathectomized animals to recover from insulin

TABLE 1

Blood sugar values in individual cats expressed as milligrams of total reducing substance per 100 cc.

The letters designate sub-groups mentioned in text

CAT NUMBER	BLOOD SUGAR IN MGM. PER 100 CC.											
	Initial 0 min.	15 min.	30 min.	60 min.	90 min.	120 min.	180 min.	240 min.	300 min.	360 min.	420 min.	480 min.
Normal												
1	87	114	71	59	55	46	*42*	51	56	73		
2	90	121	75	58	58	60	41	*37*	46	65		
3	84	134	92	56	61	57	*52*	63	89	92		
4	89	166	105	48	41	*39*	42	56	93	112		
5	94	140	72	52	39	30	*25*	39	54	68		
Lumbar sympathectomy												
(b) 6	88			51		53	*23*	84	94	91		
(b) 6	83	155	113	68		*40*	44	54	70	82		
(b) 7	87			59		47	45	*43*	61	86		
(b) 7	89	125	104	75		*34*	37	48	80	93		
Splanchnic section												
(c) 8	92	117	88	51	40	33	38	*30*	37	51	65	81
(d) 9	77	106	74	40	33	24	28	*23*	25	30	59	
(d) 10	97			35		18	20	*17*	62	60		
(c) 11	80			31	31	*23*	25	25	36	28	74	
(c) 11	91			52		*22*	29	32	35	48	86	
(e) 12	122	166	124	60	47	33	32	*25*	*25*	53	99	
(e) 12	85			33		30	27	*24*	74	111	125	
(e) 13	74			34		*15*	19	16		17	57	
(a) 14	78			25		*18*	24	29	61	72		
(a) 15	79			31		27	*22*	40	67	75		

injection. If complete sympathectomy prevents blood sugar recovery in the sense that the nerve paths which control the release of carbohydrate from the liver are inactivated then Dworkin's experiments indicate that the paths concerned do not take the course of those which control the medulli-adrenal emergency mechanism. The demonstration of the existence of such fibers would be of the greatest interest. It cannot be as-

sumed that recovery of an animal from hypoglycemic shock is dependent exclusively on the availability of glucose and that the general physical condition may be disregarded. The question is whether Dworkin's animals succumbed on account of lack of glycogenolytic activity in the liver or for other reasons. Heart action, temperature control and other factors are admittedly affected by complete sympathectomy.

CONCLUSIONS

1. After section of the splanchnic nerves in cats 2.0 units of insulin per kilo always produced shock and usually was accompanied by convulsions. Blood sugar determinations corroborate this in the sense that the splanchnics carry the impulses for prevention of excessive lowering of the blood sugar (emergency action).

2. In the animals, however, in which the emergency mechanism had been abolished a spontaneous restitution of blood sugar to normal values took place.

3. Removal of the second and third lumbar sympathetic ganglia did not produce reactions to insulin differing from the normal.

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EPINEPHRINE OUTPUT FROM THE ADRENAL GLANDS IN EXPERIMENTAL DIABETES[1]

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In a recent investigation, Rogoff and Ferrill (1936) showed that the development and course of diabetes following total pancreatectomy is not modified by reduction or suppression of epinephrine secretion from the adrenals. Further, it was observed that completely depancreatized dogs, on a constant diet and treatment with sufficient insulin to control glycosuria, sooner or later develop a reduced or suppressed epinephrine output from the adrenal glands. These observations have been confirmed in the present investigation.

We have extended these studies to determine, if possible, what factor or factors in our experimental conditions may be responsible for interference with epinephrine secretion from the adrenals. The following possibilities are apparent and constitute the basis for the studies reported here: *a*, pancreatectomy and the diabetic state of the animal; *b*, administration of insulin; *c*, altered functional state of the nervous mechanism governing epinephrine liberation. The experiments were performed on dogs. Quantitative studies on the liberation of epinephrine from the adrenals were made by the method employed extensively by Stewart and Rogoff.

Sixteen animals (table 1) were totally pancreatectomized and maintained on a diet consisting of 500 grams boiled beef lung, 100 grams cane sugar and 75 grams fresh raw beef pancreas daily. Insulin was administered in doses adequate for maintaining the daily excretion of sugar in the urine below about five grams and below about one per cent. After various periods of observation, ranging from 11 to 77 days following pancreatectomy, experiments were terminated and the animals sacrificed for determination of the epinephrine output from the adrenal glands.

Thirteen animals (table 2) were totally pancreatectomized and maintained on a diet consisting of 500 grams boiled beef lung and 75 grams fresh

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raw beef pancreas daily. They did not receive sugar, nor were they treated with insulin. After periods of observation ranging from 4 to 23 days following pancreatectomy, when evidence of severe diabetes was present, animals were sacrificed for determination of epinephrine output from the adrenals.

Ten unoperated animals (table 3) were kept on a diet consisting of 500 grams boiled beef lung and 100 grams sugar daily. Five of these dogs (numbers 1 to 5 inclusive) received insulin in doses comparable with those employed in depancreatized animals included in table 1. The other five animals did not receive insulin. Epinephrine output was determined after 22 to 64 days of observation in the first five and after 21 to 55 days in the last five animals.

It may be mentioned that in these experiments, as in those reported by Rogoff and Ferrill, preparation of the "cava pocket" for obtaining adrenal vein blood was performed under nembutal anesthesia. In a small series of control experiments we found the ordinary epinephrine output, in dogs, near the lower limit of the "normal" range more often with nembutal than was usually the case with urethane or ether anesthesia. However, the average was about the same, since the range in this series did not differ from that found by Stewart and Rogoff. Any figure that is decidedly outside the ordinary range, therefore, indicates a significant change in epinephrine output.

In normal animals, the average epinephrine output (dogs, cats) is approximately 0.0002 mgm. (0.2γ) per kgm. body weight per minute, as determined by Stewart and Rogoff (1923a). In about 85 per cent of a large series of determinations the limits were 0.0001 to 0.0003 mgm. (0.1 to 0.3γ) per kgm. These figures may be used for comparison with the figures for epinephrine output in the present series of experiments, since the determinations were made with the same method.

Table 1 shows that insulin requirement, in depancreatized animals, is not related to epinephrine secretion from the adrenals. Relatively high or low requirements may be associated with normal, reduced or practically suppressed epinephrine liberation. Furthermore, it is evident that there is a striking interference with epinephrine secretion from the adrenals, in these animals. Epinephrine output was within the range for normal dogs in only four of the sixteen animals. In six it was between one-fourth and one-tenth of the normal average and in the other six animals epinephrine output ranged between one-tenth and one-seventieth of the normal average. From a physiological standpoint, such a marked reduction in liberation of epinephrine can be considered as equivalent to complete suppression. Indeed, in some cases where the epinephrine output, as given in the table, is about one-fiftieth of the normal average or less, the actual output may have been much less if any liberation was present. Our figures,

TABLE 1

Depancreatized dogs, treated with insulin

NUMBER	SEX	DEPANCREATIZED	INSULIN REQUIRED*		BODY WEIGHT		ADRENAL WEIGHT		EPINEPHRINE OUTPUT			
			Range	Final	Initial	Terminal	Right	Left	Per minute		Per kilogram body weight per minute	
		days	*units*	*units*	*kgm.*	*kgm.*	*gram*	*gram*	*mgm.*	*γ*	*mgm.*	*γ*
1	M	33	6–38	16	9.1	8.1	1.6	1.7	0.000086	0.086	0.00001	0.01
2	M	38	4–42	6	11.7	9.65	0.91	0.88	0.000038	0.038	0.000004	0.004
3	F	53	10–40	30	10.2	8.5	0.88	0.96	0.000127	0.127	0.000015	0.015
4	M	11	10–45	45	8.8	8.5	0.61	0.55	0.0003	0.3	0.000035	0.035
5	F	60	10–45	10	8.8	7.65	0.70	0.64	0.0000286	0.0286	0.0000037	0.0037
6	M	37	5–35	10	8.5	7.4	0.50	0.47	0.001	1.0	0.000135	0.135
7	F	38	10–50	30	9.5	8.3	0.56	0.56	0.0014	1.4	0.00017	0.17
8	M	20	10–40	40	9.4	8.35	0.70	0.68	0.00034	0.34	0.00004	0.04
9	M	51	5–50	7	12.75	9.7	0.56	0.50	0.00038	0.38	0.00004	0.04
10	M	22	10–30	15	7.4	6.9	0.49	0.42	0.00013	0.13	0.000019	0.019
11	M	34	5–40	10	9.95	8.0	0.98	0.88	0.0005	0.5	0.000063	0.063
12	M	16	20–40	25	7.8	7.63	0.47	0.41	0.000057	0.057	0.0000075	0.0075
13	F	15	15–30	20	7.7	7.2	0.64	0.66	0.00127	1.27	0.00018	0.18
14	M	14	15–40	15	9.9	9.6	0.72	0.67	0.0028	2.8	0.0003	0.3
15	M	41	6–22	8	8.0	6.85	0.65	0.66	0.00028	0.28	0.00004	0.04
16	M	77	6–60	12	13.5	11.3	0.73	0.72	0.00084	0.84	0.000075	0.075

* The range of insulin given represents the minimum and maximum doses administered during the course of the experiment; the final dose was given the day preceding sacrifice of the animal for epinephrine assay.

TABLE 2

Depancreatized dogs, untreated

NUMBER	SEX	DEPANCREATIZED	BODY WEIGHT		ADRENAL WEIGHT		EPINEPHRINE OUTPUT			
			Initial	Terminal	Right	Left	Per minute		Per kilogram body weight per minute	
		days	*kgm.*	*kgm.*	*gram*	*gram*	*mgm.*	*γ*	*mgm.*	*γ*
1	F	19	9.7	7.2	0.85	0.82	0.0028	2.8	0.0004	0.4
2	M	8	10.15	8.26	0.65	0.69	0.002	2.0	0.000235	0.235
3	M	10	6.7	4.85	0.40	0.38	0.000056	0.056	0.000012	0.012
4	F	8	11.65	9.25	0.68	0.62	0.0015	1.5	0.00016	0.16
5	M	11	7.5	5.35	0.40	0.40	0.00058	0.58	0.00011	0.11
6	F	6	7.25	5.1	0.72	0.66	0.00022	0.22	0.00004	0.04
7	M	11	10.25	7.0	0.75	0.78	0.00007	0.07	0.00001	0.01
8	M	12	8.5	6.15	0.45	0.44	0.00128	1.28	0.0002	0.2
9	M	4	9.75	7.7	0.54	0.51	0.00067	0.67	0.00009	0.09
10	M	23	11.85	7.5	0.86	0.81	0.0013	1.3	0.00017	0.17
11	M	16	9.2	5.75	0.50	0.46	0.00115	1.15	0.0002	0.2
12	M	10	8.05	6.2	0.55	0.57	0.00092	0.92	0.00015	0.15
13	F	18	8.0	6.0	0.48	0.63	0.0025	2.5	0.0004	0.4

Dogs 1 and 2 were given small amounts of insulin for a few days but as they were not eating well it was discontinued.

in these cases, represent the limit of sensitivity of the test object and they indicate that not more than those amounts could have been present, if any epinephrine was being secreted.

The possibility that insulin might be responsible for the reduced or suppressed liberation of epinephrine, in these animals, was investigated by observations on epinephrine secretion in depancreatized dogs not treated with insulin. Table 2 includes thirteen of these experiments. Liberation of epinephrine was within the range for normal dogs in ten animals. In one it was reduced to one-fifth and in two dogs to one-twentieth of the normal average. It is possible that longer survival than is usual in untreated depancreatized animals might favor the development of reduced epinephrine secretion. However, the marked reduction, observed in the

TABLE 3

Unoperated dogs, receiving 100 grams sugar daily in food

NUMBER	SEX	DURATION	INSULIN INJECTED		BODY WEIGHT		ADRENAL WEIGHT		EPINEPHRINE OUTPUT			
			Range	Final	Initial	Terminal	Right	Left	Per minute		Per kilogram body weight per minute	
		days	*units*	*units*	*kgm.*	*kgm.*	*gram*	*gram*	*mgm.*	*γ*	*mgm.*	*γ*
1	F	22	6–14	8	10.52	10.5	0.52	0.69	0.000126	0.126	0.0000125	0.0125
2	M	51	6–24	12	9.52	10.7	0.59	0.64	0.00235	2.35	0.00022	0.22
3	M	39	6–26	26	12.05	12.55	1.06	0.91	0.00003	0.03	0.0000024	0.0024
4	M	64	8–40	40	9.2	10.9	0.65	0.55	0.000034	0.034	0.000003	0.003
5	M	44	4–24	24	8.1	8.2	0.76	0.74	0.00006	0.06	0.000008	0.008
6	M	21	0	0	11.35	10.2	0.56	0.50	0.00112	1.12	0.00011	0.11
7	M	48	0	0	12.55	12.95	0.68	0.65	0.0055	5.5	0.00042	0.42
8	M	55	0	0	13.4	14.4	0.64	0.61	0.000065	0.065	0.0000045	0.0045
9	F	46	0	0	9.05	10.0	0.79	0.84	0.0024	2.4	0.00024	0.24
10	F	41	0	0	8.7	10.3	0.47	0.45	0.0003	0.3	0.00003	0.03

The first 5 animals in the table received insulin, the others did not.

three animals of this group, indicates that insulin is not primarily responsible for the interference with epinephrine secretion.

It appears that the diabetic state of the animal is the principal factor which leads to reduced or suppressed liberation of epinephrine. Although the marked effect on epinephrine secretion was found in a larger proportion of the animals treated with insulin, a significant number of the untreated animals also were similarly affected.

The evidence indicating that the diabetic state of the animals was primarily responsible for interference with epinephrine secretion led us to investigate the influence of prolonged administration of relatively large amounts of sugar, in non-depancreatized animals, with and without daily injections of insulin. As shown in table 3, we obtained results similar to those in the pancreatectomized animals. Of the five unoperated animals

that received insulin in addition to sugar, only one showed a normal epinephrine output. In the other four dogs, epinephrine liberation was from one-twentieth to about one-hundredth of the average for normal animals. In the other group of five animals, not receiving insulin, three were found to have a normal epinephrine output, one had about one-eighth and one about one-fiftieth of the normal average. In so small a series of experiments, it is significant that epinephrine liberation was markedly reduced in two out of five animals.

Thus far, we have no information that might explain the mechanism whereby the adrenal medulla is so strikingly influenced by the diabetic state. The observations that reduction or suppression of epinephrine secrètion was more commonly found in insulin-treated diabetic animals, and that ingestion of excess of sugar alone over a prolonged period can similarly influence the liberation of epinephrine, suggest the probability of a rôle played by the liver. This and other possibilities remain for further investigation.

We made observations to determine whether the integrity of the splanchnic nerve supply to the adrenal medulla is altered and found that stimulation of the splanchnic nerve is capable of increasing the epinephrine output up to or above the normal level, in those animals that show a marked reduction in output.

Discussion. These experiments support the conclusions by Stewart and Rogoff (1923b) and Rogoff and Ferrill (1936, 1937), that suppression of epinephrine secretion from the adrenals does not influence the development, intensity or course of diabetes following extirpation of the pancreas. We believe that the experimental evidence for the view to the contrary is untenable. Much of it rests on results obtained with animals that were not in sufficiently good condition to permit satisfactory conclusions. Hitherto, quantitative studies on liberation of epinephrine were lacking.

It is significant that such quantitative observations prove not only that suppression of epinephrine secretion, by surgical intervention with the adrenals, is without influence on the development and course of experimental pancreatic diabetes, but that the diabetes itself leads to diminished or suppressed liberation of epinephrine. This observation is of special importance in view of the clinical practice of roentgen or surgical intervention with the adrenals as a therapeutic measure in human diabetes. Not only does such a procedure involve great risk of damage to the indispensable adrenal cortex, but it may be expected to aggravate an already existing pathologic condition.

SUMMARY

1. Additional evidence is presented showing that the development, severity and course of experimental pancreatic diabetes are not influenced by reduction or suppression of epinephrine secretion from the adrenals.

2. Diabetic animals, not subjected to surgical interference with the adrenals, sooner or later develop reduced or suppressed secretion of epinephrine. This appears to be due primarily to the diabetic state.

3. Electrical stimulation of the splanchnic nerve is capable of increasing the epinephrine output up to or above the normal level, in those animals that show a marked reduction in output.

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THE INFLUENCE OF INCREASED METABOLISM ON β-HYDROXYBUTYRIC ACID UTILIZATION[1]

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The recent evidence of Friedeman, Somogyi and Webb (1), Burn (2), Chaikoff and Soskin (3), Friedeman (4), Mirsky and Broh-Kahn (5) and others indicates that neither glucose nor insulin administration can increase the utilization of ketone bodies by the tissues of the normal animal or those of the hepatectomized-depancreatectomized animal. We have recently interpreted this to mean that the effect of glucose and of insulin in diabetic or non-diabetic ketosis is due not to an increased ketolysis but to an inhibition of fat oxidation in the liver, i.e., antiketogenesis (5)(6). However, our data did not exclude the possibility that under normal conditions or in the absence of the liver the utilization of ketone bodies is still dependent upon the simultaneous oxidation of glucose. In view of our observations that the utilization of carbohydrate is markedly accelerated in experimental hyperthyroidism (7) we studied the effect of an increase in metabolism on the utilization of injected ketone bodies in order to find whether or not there exists some quantitative relationship between the utilization of sugar and that of ketones.

While this study was in progress, Barker (8) reported that an increase in the metabolism of the depancreatized dog consequent to dinitrophenol or thyroid administration resulted in an increase in the oxidation of fat as judged from respiratory quotient data. Nevertheless, in no instance did he observe an increased ketonuria commensurate with that which was expected from the calculated increased amounts of fat burned. No explanation for this discrepancy was offered. The data which we have obtained in the present study suggest an adequate explanation for Barker's results.

METHODS. In accord with our previous work, the removal of β-hydroxybutyric acid from the blood was used as an indicator of ketone utilization. From 800 to 900 mgm. of the sodium salt of the racemic acid were administered intravenously to all animals in the form of a neutralized, 10 per cent

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solution. Subsequent to the injection, an interval of 15 minutes was allowed to elapse for diffusion and mixing. At the end of this time, arterial blood samples were drawn from the exposed femoral artery and at half-hourly intervals thereafter. The whole blood was analyzed for "total ketones" by the Van Slyke and Fitz method and for sugar by the Somogyi modification of the Shaffer-Hartmann procedure.

Two methods of increasing metabolism were employed. To two lots of animals, 20 mgm. per kilogram body weight of 2,4-dinitrophenol were administered subcutaneously 30 minutes prior to the injection of the sodium β-hydroxybutyrate. Another set of animals was rendered hyperthyroid by the daily, oral administration of 10 grains of desiccated thyroid until a 20 per cent loss in body weight was noted.

As in our previous study (5), male white rabbits of approximately 2 kgm. body weight were employed. Five groups of experiments were performed,

TABLE 1

Influence of increased metabolism on β-hydroxybutyric acid utilization

EXPERIMENT	NUMBER OF ANIMALS	BLOOD SUGAR			BLOOD "TOTAL ACETONE"*		
		Initial	30 minutes	60 minutes	Initial	30 minutes	60 minutes
		mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
A. Nephrectomized normal controls	6	96	135	200	69.3	43.0	25.8
B. Nephrectomized dinitrophenol	6	112	149	198	67.8	27.7	9.3
C. Eviscerated controls	6	117	97	74	67.9	41.0	22.9
D. Eviscerated dinitrophenol	6	114	85	47	67.3	25.8	11.2
E. Eviscerated hyperthyroid	6	91	75	38	63.4	27.4	8.6

* Expressed as β-hydroxybutyric acid.

each group comprising a minimum of six animals. Two groups consisted of nephrectomized rabbits and three of completely eviscerated animals prepared as previously described (7). All the studies were carried out while the animals were under nembutal anesthesia.

The rate of ketone utilization was observed in the following sets of animals: A, nephrectomized, normal; B, nephrectomized, dinitrophenol; C, eviscerated, normal; D, eviscerated, dinitrophenol; and E, eviscerated, hyperthyroid. In view of the fact that hyperthyroidism is frequently associated with a ketosis, and since the liver is known to be the source of ketone body production (9), it was deemed unnecessary to complicate matters further by attempting to study the rate of ketone utilization in the intact, hyperthyroid preparation.

RESULTS. The data are summarized in table 1. Using the nephrectomized-normal and the eviscerated-normal as controls, it is apparent that an

increased metabolism consequent either to dinitrophenol administration or to hyperthyroidism is associated with a marked increase in the rate of disappearance of the ketones from the blood. This disappearance has been demonstrated to be an adequate criterion for oxidation of these substances by the extrahepatic tissues (4)(5). A comparison of the nephrectomized groups A and C with the eviscerated groups B and D affords further evidence that the liver is incapable of the oxidation of ketone bodies (5).

It has already been demonstrated that hyperthyroidism produces an increase in the rate of carbohydrate utilization by the extrahepatic tissues (7). It is apparent from these results that dinitrophenol has the same effect. Thus, whereas in the eviscerated controls, the blood sugar fell from an initial value of 117 mgm. per cent to a value of 74 mgm. per cent, in the same period, the average value in the eviscerated dinitrophenol animals fell from 114 to 47 mgm. per cent.

It is of interest to note the progressive hyperglycemia that follows the injection of hydroxybutyrate into the nephrectomized dinitrophenol rabbits as well as in the nephrectomized controls. This can in no way be interpreted as due to a suppression of carbohydrate oxidation by the extrahepatic tissues inasmuch as the eviscerate-dinitrophenol animals exhibit a marked increase in carbohydrate utilization. It is probable that the hyperglycemia is due to an increased glycogenolysis.

Discussion. It is evident from these results that an increased metabolism elicits an increased rate of utilization of ketone bodies by the extrahepatic tissues. It has been previously emphasized that the degree of ketonemia and ketonuria is the resultant of two factors: 1, the rate of ketone formation, and 2, the rate of ketone utilization. If the latter is unknown, mere determination of the degree of ketonemia or ketonuria is insufficient for the estimation of the degree of ketogenesis.

Implicit in Barker's (8) calculations is the assumption that ketones accumulate in the blood and urine of the depancreatized dog because they are incapable of further oxidation. However, as Chaikoff and Soskin (3) demonstrated, the diabetic organism utilizes ketones at a normal rate but these substances accumulate solely in amounts that represent the balance between their rate of formation by the liver and their rate of utilization by the extrahepatic tissues. Even if it be true that the increased metabolism consequent to dinitrophenol or thyroid administration is solely at the expense of fat, and even if it were possible to calculate the amount of ketones that should be produced by this increased combustion of fatty acids, our data indicate that the increased utilization of ketones under such conditions will prevent their accumulation in the blood and urine to the extent anticipated from the breakdown of their precursors.

Shaffer and others from their "in vivo" and "in vitro" studies concluded

that there exists a definite ketolytic ratio for glucose. This ratio has been variously stated to range from 1.0 (10) to 2.0 (11). Analyzing our data, we find that in the eviscerated control group (C) the blood sugar dropped an average of 43 mgm. per cent in the period during which the ketones fell 45 mgm. per cent. From the molecular weights of glucose and β-hydroxybutyric acid, it follows that the average molecular ratio of ketones burned to glucose oxidized is 1.80 for the normal group. Similarly a ratio of 1.46 is observed in the dinitrophenol group (D) and 1.79 in the thyroid-fed group (E). These figures are well over the ratio of 1, but slightly below the ratio of 2. However, examination of individual experiments reveals instances where ratios over 3 were observed. Other studies now in progress lead us to conclude that any "ketolytic" ratio is fortuitous.

These considerations suggest that ketogenesis may be proceeding without any detectable ketosis if the rate of ketone utilization equals or exceeds the rate of formation. Furthermore, the degree of ketonuria may be expected to offer, at the best, only a qualitative index of the rate of ketogenesis (i.e., fat oxidation).

These considerations suggest very little, if any, relationship between carbohydrate oxidation and ketolysis, and are in accord with the hypothesis that glucose exerts its antiketogenic action solely by virtue of its ability to suppress fat oxidation in the liver (5).

CONCLUSIONS

1. An increased metabolism consequent to dinitrophenol or thyroid administration markedly increases the rate of ketone utilization by the extrahepatic tissues. The utilization of glucose is likewise affected.

2. The relationship between carbohydrate utilization and ketone body utilization has been studied and no evidence of a definite "ketolytic" ratio *in vivo* could be demonstrated.

3. The effect of an increased metabolism on ketosis is dependent upon the balance between the increased rate of ketone formation and the increased rate of ketone utilization.

4. The effect of glucose in ketosis is attributed solely to an inhibition of fat oxidation in the liver.

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THE EFFECTS OF TOTAL PLASMAPHERESIS AND PROTEIN REGENERATION UPON THE AGGLUTINATION TITRE IN DOGS IMMUNIZED AGAINST B. TYPHOSUS

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Stanbury, Warweg and Amberson (1936) have recently described a method of total plasmapheresis by which all but the last trace of the plasma proteins may be removed from the blood stream. The normal blood is removed from the body by long perfusion with a gum-Ringer-Locke solution in which are suspended washed erythrocytes from a donor of the same species. The animals survive indefinitely without shock or edema, and the normal serum proteins are shortly replaced, while the gum disappears from the blood stream.

It seemed to us that this technique might furnish an opportunity to study the antibodies of the blood from a new point of view. It is well known that immune reactions are intimately associated with the serum proteins, and there is much evidence that antibodies are developed in close relation with the globulin fraction, or may even represent modifications of this protein itself. Crucial experiments, however, are all too few. We have therefore attempted to study the effect of total plasmapheresis upon the immune titre of dogs immunized against B. typhosus.

METHODS. Healthy dogs weighing from 15 to 20 pounds were selected. After a preliminary observation during which the diet was standardized, control blood samples were taken for the determination of plasma proteins and natural immune titre. Immunization was accomplished by the usual method of intravenous injection of prepared typhoid vaccine, followed later by injections of suspended living organisms.

After immunization, a short interval of time was permitted to elapse before the animals were subjected to plasmapheresis, in order that the immune titre might reach a plateau. The animals then were subjected to plasmapheresis by the method previously cited. Samples of blood were taken immediately before and after plasmapheresis (which requires approximately one and one-half hours) and again at thirty minutes, and four and one-half hours after completion of the operation; thereafter at less frequent intervals. Plasma proteins were determined by the Kjeldahl

TABLE 1

Immune titre before and after plasmapheresis

	DOG 1	DOG 2	DOG 3	DOG 4	DOG 5	DOG 7	DOG 9	DOG 11	DOG 12	DOG 14	DOG 15
Normal titre	80	80	80	40	80	80	80	40	40	40	80
Immunization begun	Nov. 27	Nov. 27	Nov. 27	Jan. 20	Jan. 20	Feb. 7	Feb. 7	Apr. 7	Apr. 7	Apr. 17	Apr. 17
Maximal titre	20,480	20,480	20,480	2,560	1,280	640	2,560	11,240	5,120	5,120	10,240
Titre immediately before plasmapheresis	2,560	10,240	20,480	640	640	640	640	2,560	5,120	1,280	
First plasmapheresis date	Jan. 2	Jan. 9	Jan. 15	Feb. 20	Feb. 20	Mar. 11	Mar. 12	May 11	May 4	May 20	Control Apr. 24
Titre immediately after plasmapheresis	40	0	80	20	0	20	20	40	40	40	640
Titre thirty minutes after plasmapheresis	40	40	160*	20	20	40	20	40	80	40	
Titre 4½ hours after plasmapheresis	40	320		40	20*	80*	40	80	160	160	
Titre 15 to 20 hours after plasmapheresis	160*	320		80			160*	160	640	320	May 1 2,560
Maximal return of titre		5,120 (10 days)		1,280 (6 days)				640 (4 days)	2,560 (10 days)	640 (9 days)	
Titre immediately before second plasmapheresis		2,560		640				640			
Second plasmapheresis date		Jan. 30		Mar. 5				May 25			
Titre immediately after second plasmapheresis		40		0				20			May 9 10,240
Titre thirty minutes after second plasmapheresis		40		20				20			
Titre 4½ hours after second plasmapheresis		80		80				80*			May 14 10,240
Maximal return of titre		160 (10 days)		640 (14 days)							
Titre 47 days after 1st immunization injection											June 3 2,560

* Died.

method after fractionation as described by Peters and Van Slyke (1932). The agglutinating titre was determined by the falling dilution method.

This paper includes data accumulated from eleven dogs. One was used as a control and ten were subjected to plasmapheresis. Of these ten, five completely recovered from total plasmapheresis. Five dogs died between 5 and 15 hours after the operation; data from these dogs are included in our tables, since, so far as they go, they are in accordance with

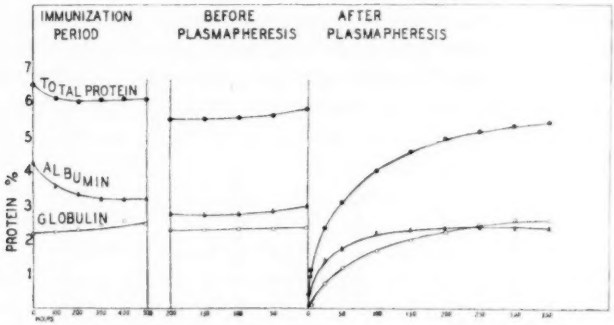

Fig. 1. Average values for total protein, albumin, and globulin for five immunized dogs subjected to seven plasmaphereses.

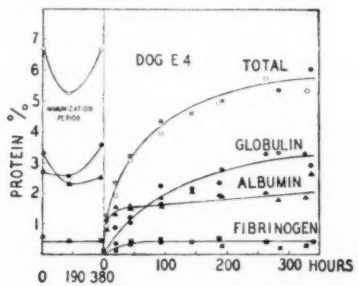

Fig. 2. Replacement of plasma proteins after two successive plasmaphereses in a single dog immunized against *B. typhosus*. ○○△□ after first plasmapheresis. ⊗●▲■ after second plasmapheresis, 14 days later.

our other experiments. Out of three attempted, two animals survived not only a first plasmapheresis, but also a second, and remained in good health until the end of the study. The third dog survived a second plasmapheresis for slightly longer than 5 hours.

Results. Table 1 clearly shows that by this method we were successful in two instances (dogs 2 and 5) in removing all demonstrable antibodies from the blood by the first plasmapheresis, and in the remaining

dogs reduced them to a level equal to or less than the original natural titre. An increase in the titre, although slight, was definite within four and one-half hours after plasmapheresis, and in a few animals the increase appeared in thirty minutes. This table further demonstrates that immune bodies reach a new maximal titre four to ten days after plasmapheresis. This new maximum in most cases is slightly less than the level before plasmapheresis, but the control also shows a slight decline with time.

In the dogs surviving a second plasmapheresis (2, 4, 11) a return of immune bodies to pre-plasmapheresis level was observed only in dog 4. In this animal, all the demonstrable immune bodies were completely removed by this second operation. A similar rate of return was in progress in

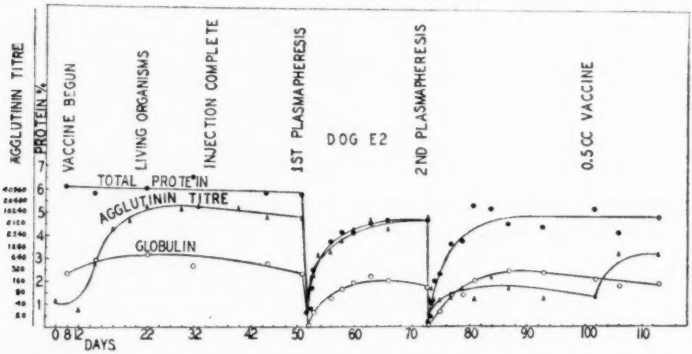

Fig. 3. Relationship of immune titre to total serum protein and to globulin in a single dog immunized against *B. typhosus*, and subjected to two successive plasmapheresis. Seven hundred hours after the second plasmapheresis 0.5 cc. of *B. typhosus* vaccine was given intravenously.

dog 11 up to the time of his death. In dog 2, the immune titre failed to reach the pre-plasmapheresis level. This variation may be partially explained by the fact that the time interval between immunization and the second plasmapheresis was greater in dog 2 than in dog 4 (table 1). Dog 2, however, responded to a single injection of 0.5 cc. of vaccine by an increase in titre from 1:80 to 1:1280 in ten days, showing that the antibody mechanism was not exhausted by this experimental procedure (see fig. 3).

The results on plasma protein behavior in the immunized dog before and after plasmapheresis are shown in figure 1, which represents the average of the data taken from five dogs with a total of seven plasmaphereses. Total proteins showed a small decrease in the early part of the immunization period. A similar decline occurred in the albumin fraction, indicating

that the protein loss at this time was albumin. There was also a slight rise in total globulin during this period. These two factors thus caused the A/G ratio to approach nearer to unity. This average A/G ratio reached a value of about 1.3 which persisted until plasmapheresis; before immunization the A/G ratio had an average value of 2. In several individual cases, it fell below unity as illustrated by figure 2. This is in keeping with the generally accepted view of globulin behavior during immunization (Hewitt, 1934; Rubenstein and Fischer, 1934; Wohlfeil and Heymer, 1934; Doerr and Hallauer, 1926; Mudd, 1932, etc.).

After plasmapheresis the albumin reappeared more rapidly than the globulin, but the quantity of globulin finally reached that of albumin and eventually surpassed it (fig. 1). In experiments with normal dogs, Stanbury, Warweg and Amberson (1936) have not noted such a reversal. We have observed that eventually the albumin-globulin ratio again rises above unity, returning to the value characteristic of the immunized animal.

These experiments clearly offer an excellent opportunity to correlate the immune body return with the plasma protein reappearance. A typical result is illustrated by figure 3. The comparison of immune titre return (fig. 3 and table 1) with globulin and albumin reappearance (figs. 1 and 2) shows that globulin and immune titre returned at similar rates, reaching a maximum in about ten days, whereas albumin had returned to its highest level about the beginning of the fifth day.

Discussion. The effects of hemorrhage, with or without transfusion, on the antibody content of blood have been studied by several authors. Roux and Vaillard (1893) found that the concentration of antibodies in serum of highly immunized animals may show no appreciable change after repeated and massive bleedings. Friedberger and Dorner (1905) state that there is a stimulating effect upon antibody production by blood withdrawal, either before or immediately after an immunizing injection. Hektoen and Carlson (1910) bled immunized dogs dry from the carotid artery and immediately transfused them with the blood from normal animals. The antibody titre was lowered, but the new formation of antibodies proceeded in a "typical manner," the curve in some cases reaching a very high mark. They also attribute their results to the effects of hemorrhage upon the hemopoietic system, despite the fact that the dogs received approximately as much blood as they had lost, and the fact that such bleeding is generally known to remove not more than 60 per cent of an animal's blood. The above results, attributed to stimulation of the hemopoietic system by hemorrhage, cannot be exactly compared with a plasmapheresis procedure, where withdrawn blood is immediately replaced by an equal quantity of transfusate containing a normal quantity of hemoglobin, but devoid of plasma proteins.

A different type of experiment was performed by Okamato (1932) who found that the transfusion of whole blood stimulated the production of various antibodies in rabbits despite the fact that there had been no preceding hemorrhage. Saline injections had a similar but lesser effect.

Freund and Whitney (1928) conducted experiments somewhat similar to our own, using immunized rabbits. They withdrew 30 cc. at a time for about fifteen bleedings, injecting after each bleeding a mixture of defibrinated normal blood and 5 per cent acacia in Locke's solution, in a ratio of 100 to 200 cc. of blood in 300 cc. of acacia solution. Their animals died at the termination of their experiments. These acute experiments were directed to the determination of the total amount of antibodies capable of being washed from the animal, and were not concerned with their regeneration. The authors concluded that more antibody could be obtained by such a procedure than calculations showed were present in the circulating blood alone, which indicates the existence of a certain amount of storage in the body. However, our results permit a fairly safe conclusion that storage alone will not entirely account for the return of the antibodies, especially after a second plasmapheresis. It seems much more probable that following the development of an immune process, the antibody mechanism may continue to function for a time even after the stimulant has been removed. It seems unlikely that the antigenic factor is still actively functioning per se after a lapse of considerable time and two complete plasmaphereses. This view receives support from the fact that dog 2 lost in part its ability to regenerate antibodies after a second operation. But that this was not due to damage to the antibody-forming mechanism is clearly evidenced by the fact that a small dose of antigen was able immediately to restart the machinery (fig. 3).

We may, therefore, conclude that even if part of the immune body return is essentially a redistribution of previously accumulated material, it is improbable that all of this restoration can be accounted for on this basis, as is shown especially by dogs 2, 4, 11 following a second complete plasmapheresis. Thus our experiments add support to the view that antibodies are formed in the tissues and continue to be formed there for some time after the process of immunization has terminated. Moreover, as the globulin regeneration and immune titre return occurred at similar rates, the experiments also support the view that the antibody formation is closely associated with the same mechanism that produces globulin.

SUMMARY

1. The method for complete plasmapheresis has been applied to a study of the regeneration relationship between plasma proteins and immune titre in dogs.

2. Demonstrable antibodies can be temporarily removed from the blood by this technique.

3. The antibody titre is capable of returning to its previous level after a first and even after a second plasmapheresis.

4. In most animals, however, the antibody titre does not return completely after plasmapheresis. This partial failure to recover is apparently due mainly to the slow disappearance of the immune state, which is also evident in the control.

5. The regeneration of antibodies parallels the regeneration of globulin, which suggests that the antibodies are closely related to the globulin fraction of the plasma proteins.

We wish to express our appreciation for funds received from the Tennessee Pharmaceutical Association and the Penrose Fund of the American Philosophical Society. We are indebted to the Eli Lilly Company of Indianapolis for supplying the acacia. The vaccine and cultures were generously supplied by the Tennessee Department of Public Health through the Memphis and Shelby County Health Department.

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THE SIGNIFICANCE OF SUBNORMAL RESPIRATORY QUOTIENT VALUES INDUCED BY CONTROLLED FEEDING IN THE RAT

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An investigation of the effect of controlled feeding on the metabolic rate of rats has brought to light several interesting phenomena, the most important of which concerns the respiratory quotient.

METHODS. As subjects for the experiments, only healthy and sexually mature male and female rats were used. Some were of strains inbred in our colony, others were crossbred from these strains.

The feeding was controlled by allowing the animals to eat but once a day for periods varying in length from one to five hours. Feeding was done either by hand or by an automatic feeding device consisting of a shutter, operated by an electric timer, which controlled access to the food. The diet consisted of dog chow pellets or food similarly prepared.

An important factor in this method of controlled feeding is that, although the feeding period occurred at a regular time daily, the time of day at which the period occurred was of no significance in the results and was fixed only with reference to convenience.

The animals became conditioned to this regime usually by two weeks. Initially the animal normally loses some weight, but a successfully conditioned animal regains this weight by eating sufficiently only during the allotted period. Only animals which had regained the initial loss and resumed normal growth were used in these experiments.

Measurements of the gaseous exchange were made with the author's apparatus, which has been described previously (Werthessen, 1937). This apparatus is designed for prolonged experiments and gives continuous readings of rate of oxygen consumption. Carbon-dioxide production is measured over at least a forty-five minute period. Rate of CO_2 production is equal to $\frac{\text{cc. produced}}{\text{Time of production}}$. All experiments were conducted at a constant external temperature of 25°C.

RESULTS. Before discussing the results pertinent only to the respiratory quotient, it may be well to point out one general result not discussed here but to be reported in detail in a later paper. This is the fact that con-

trolled feeding seriously affects the diurnal variation described by Horst, Mendel, and Benedict (1934). They found that the basal rate was from 13 to 31 per cent higher from 2:00 a.m. to 10:00 a.m. than from 10:00 a.m. to 4:00 p.m. After 4:00 p.m. the rate rose again. Controlled feeding may induce a variation with a difference as high as 700 per cent between the highest and lowest values obtained (see fig. 1).

At first, as in experiment *A*, it was found that in general the animals which had been conditioned for a short period, not exceeding a month,

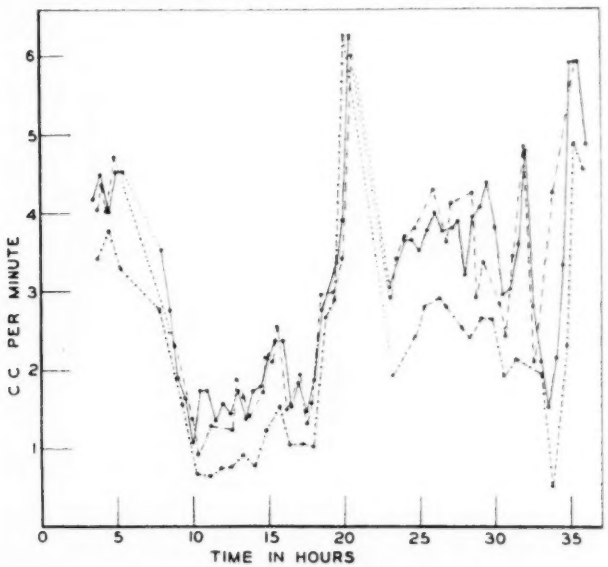

Fig. 1. The variation in rate of O_2 consumption and CO_2 production as a function of the time after eating as found in experiment *A*. Conditioning period less than 3 months. End of feeding period occurred at 0 time—No food given during experiment. ——— = O_2 consumption averaged over half-hour intervals. · — · — = O_2 consumption during quiescent periods. − + − + = rate of CO_2 production.

showed the type of R.Q. curve shown in figure 2. In this graph the R.Q. values found at various consecutive points during the experiment have been plotted against the time elapsed since the end of the feeding period. The resulting curve shows an unquestionable cycle in R.Q. values. The lowest value found is 0.35. The average value for the post-absorptive period is 0.73. The values at the beginning of the experiment are high. A decrease in value occurs during the first half of the experiment. After the lowest point has been reached the values rise to normal and above.

Experiment A therefore shows clearly that some mechanism is in control which allows the occurrence of very low R.Q. values and yet maintains an average R.Q. of such a value as to indicate that no unusual substance is the basis of the metabolic reactions.

In experiment *B* we find another result, not as common in our data for the shorter training period (fig. 3). Here the fall is interrupted by a sudden brief outpouring of CO_2 with little change in O_2 consumption. Thus we obtain what may be called a compensating value. When the animals have gone through a longer conditioning period of several months,

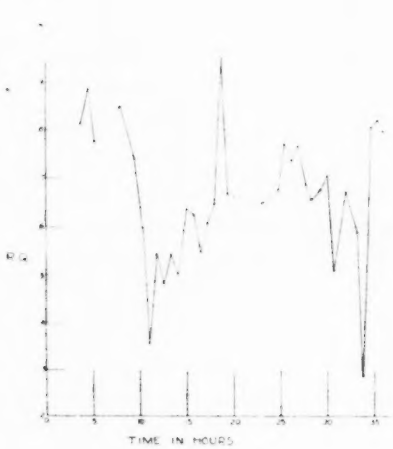

Fig. 2

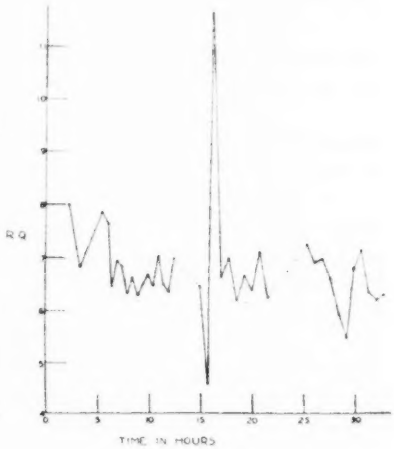

Fig. 3

Fig. 2. The change in R.Q. as a function of time after eating as found in experiment *A*. Conditioning period less than 3 months. End of feeding period occurred at 0 time—No food given during experiment.

Fig. 3. The change in R.Q. as a function of time after eating as found in experiment *B*. Conditioning period was less than 3 months. End of feeding period occurred at 0 time—No food given during experiment.

little evidence of a cycle is found, as shown in figures 4 and 5, although occasionally a good cycle is found (expts. 35, 39, 54). The beginning of a cycle is seen; but immediately after the lowest value an unusually high one occurs. The average of these values is again a normal R.Q. Here low values are not as low as in the data for the shorter training periods. High values far above 1.00 and approaching 2.00 are common.

Since the results in these experiments disagree with the data of literature on the subject, check experiments were necessitated to determine the validity of the observations.

The first step toward this end was a complete recalibration of the apparatus used. It was found that in neither the recording ammeter nor the Wheatstone bridge was there any error of sufficient magnitude to account for the apparently aberrant low and high values. The CO_2 partial pressure in the respiration chamber was shown to be constant within the error

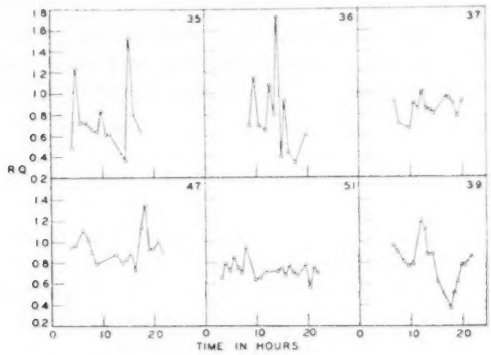

Fig. 4

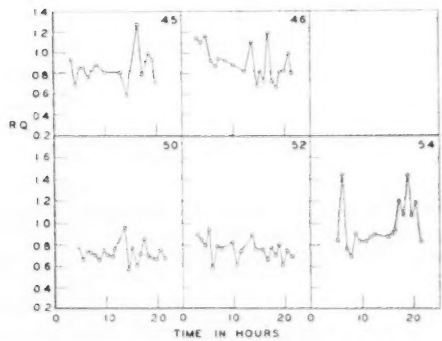

Fig. 5

Figs. 4 and 5. The change in respiratory quotient as a function of the time after eating as found in animals conditioned for 6 months. End of feeding period occurred at 0 time—No food given during experiment.

of the gas-analysis apparatus used. This lack of variation showed that this was not the source of the questioned values.

As a further check, experiments were done on unconditioned animals to see if normal values could be obtained with the same apparatus. Normal values were always found in such experiments. Such values, for example,

run: 0.94, 0.90, 0.82, 0.80, 0.78, 0.78, 0.76, 0.71, 0.77, 0.70 when an unconditioned animal is examined. The first reading is at 3 hours after eating, the last at 14 hours. The readings are evenly spaced. Such an experiment shows the decline in R.Q. value associated with the approach to the post-absorptive condition.

The final and most convincing check of the validity of the questioned values was made by using standard equipment on a trained animal. For this experiment a male rat trained for six months was used. The analyses were made with standard Carpenter-Haldane apparatus by Messrs. William and Frank Consalazio of the Fatigue Laboratory of the Harvard Business School.

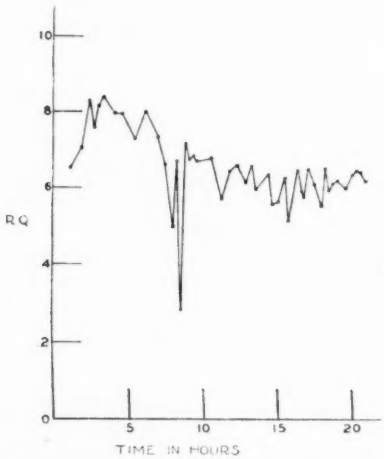

Fig. 6. The variation in R.Q. as function of time after eating as found when the experiment was performed by other observers using standard apparatus. Conditioned for 6 months. End of feeding period occurred at 0 time—No food given during experiment.

Moistened outside air was passed through a temperature regulating coil into a thermostated jar, containing the animal, at a temperature of 25°C. The rate of flow was constantly measured. After the air had passed over the rat it was collected and samples were taken for analysis. All the measurements were made immediately upon taking the samples.

As a check experiment these data corroborate the findings already presented. The data are presented graphically in figure 6. It should be noted that these data include the lowest R.Q. value, 0.27, found in all these experiments. The curve does not show a marked cycle, but it does show that values below 0.6 can be obtained with properly functioning standard apparatus.

For argument let us assume that the lowest values in this check experiment are wrong. There are still sufficient points after the R.Q. of 0.27 to serve as a check without them. Since outside air samples were taken at reasonable intervals, analyzed and found to give normal values, it cannot be said that the general low level was due to a constant error in the apparatus.

There appear to be four possibilities with regard to the general level of R.Q. values here presented. 1, the values are too low, due to faulty technique; 2, the low R.Q. values may be explained by CO_2 storage; 3, some unknown factor is operating to produce spuriously low values; 4, the results are real and indicate the nature of the metabolic process occurring.

With regard to 1, reference may be made to what has been said above as to the checks made of the apparatus. Further, the fact that a control experiment, performed with standard apparatus by expert observers from another laboratory, gave results in essential agreement with those obtained by the author, seems to render the possibility of faulty technique remote.

The isolated occurrence of excessively low R.Q. values (0.5) may perhaps be explained as the result of temporary CO_2 storage. Such an explanation cannot, however, account for the general low levels of R.Q. maintained over long periods. Let us assume that all R.Q. values below 0.7 represent periods of CO_2 storage. Then in experiment *A*, calculation shows that the average concentration of CO_2 in the whole animal must be about 150 volumes per cent before normal values are obtained again. Since a CO_2 concentration in the blood of about 80 gives rise to pathlogical effects, we can consider that CO_2 storage is ruled out.

Relative to 3, nothing positive can be said until some suggestion as to the nature of this hypothetical factor is offered. All that can be said is that no such suggestion has yet been forthcoming.

Discussion. In what follows we shall assume that the R.Q. values obtained are real, and are to be interpreted as representative of processes actually occurring in the organism.

The respiratory quotient has been used as an index of the general nature of the metabolites of a metabolic process. An R.Q. of 1.00 has been considered representative of glucose oxidation, 0.8 of protein oxidation and 0.7 of fat oxidation.

It can be considered an unquestionable fact that when any of these three classes of foodstuff are oxidized completely, the R.Q. representative of their oxidation will be obtained.

There are, however, two methods of oxidation possible. The first is the complete, immediate oxidation. That is, once a molecule of a substance is attacked, nothing results from the reaction but CO_2, water and energy except where proteins are concerned and urea is also formed.

The second type of oxidation is accomplished by a splitting of the larger molecule of the substrate into smaller simpler substances. The simpler substances are attacked in turn and the final product is again CO_2, water, and energy, with urea when protein oxidation is occurring. The overall R.Q. is that of the initial substrate. Steps in the process have individual R.Qs. This second type of oxidation is a chain reaction system. It involves an enzyme train.

If we assume that oxidative processes in the mammal follow our first method, there should be found no R.Q. values below 0.7 which cannot be explained by abnormal CO_2 production.

If we assume the chain reaction system, then values below 0.7 should be found under special conditions. These conditions may involve either the stopping of the reactions at some point in the chain, or the drawing out of the time required for the complete oxidation. The time must be extended far enough to allow observation of the separate steps.

In these experiments no attempt was made to stop the reactions at some point.

Thus we must assume that controlled feeding in some manner exposes the chain reactions by slowing down the metabolic processes. If the metabolic process is slowed down we should expect that the oxidation would be eventually completed. If it is completed then high R.Q. values approaching 1.00 should be found when the simplest substances are oxidized. Values below 0.7 and approaching 0.2 should be found for the initial steps of the reaction. These high values may be approached gradually or spasmodically.

But in all cases the average R.Q. should be the R.Q. of a normal rat in the post absorptive state. We find the average R.Q. to be normal in most of our experiments. In those where it is below normal the presumption can be made that the experiment was not continued long enough to obtain the higher values.

We can then on the basis of our findings and the argument given, come to the conclusion that direct oxidation of foodstuffs does not occur in the rat.

Metabolism is therefore based on a chain reaction system. Fats and proteins are presumably split and converted into complex carbohydrates. These carbohydrates are converted into either glucose or some similar substance.

If the processes are sufficiently deranged it is not improbable that excessive conversion of complex into simpler molecules may occur. Reconversion could then follow. The findings of R.Q. levels indicative of such derangement would strengthen our case.

In animals trained for long periods we have found R.Q. values so high

that the only explanation can be the conversion of some complex carbohydrate into fat (figs. 4 and 5, expts. 35, 36, 54).

SUMMARY. Rats were conditioned to eat for a short period of time beginning once every 24 hours. An initial weight loss when placed on this regime is followed by recovery in two weeks.

Animals which had been on the regime from 1 month to 6 months were used as experimental subjects. Oxygen consumption and CO_2 production were measured.

Respiratory quotient values for an animal so conditioned were found to vary between 0.3 and 1.7. Check experiments, demonstrating the validity of the findings, were performed.

CONCLUSIONS

1. Controlled feeding induces a derangement of the time course of the oxidation of substrates.
2. The derangement consists of a slowing down of the rate of reaction in the chain of processes involving CO_2 production.
3. The decrease in rate exposes the various steps in the oxidations of the substrates.
4. By finding R.Q. values ranging between 0.27 and 0.7 for low values and between 1.0 and 1.70 for higher values, the conclusion is drawn that a chain reaction system is the mechanism of oxidation.
5. Steps in the oxidative processes involve the conversion of fat into a complex carbohydrate, the conversion of the carbohydrate into simple substances of the glucose type.

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THE EFFECT OF REGENERATION OF THE NERVE SUPPLY ON THE SENSITIVITY OF THE DENERVATED NICTITATING MEMBRANE TO ADRENINE

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The increased response of smooth muscle to adrenine after sympathetic denervation is now well known. The "paradoxical pupillary reaction," studied by earlier investigators (Anderson, 1903; Meltzer and Auer, 1904), lacked adequate explanation until Elliott (1905) suggested that the phenomenon was an expression of increased sensitivity of smooth muscle to injected or secreted adrenine. More recently, Hampel (1935) studied the time-course of the development of sensitivity to adrenalin in the nictitating membrane of the cat after degeneration of preganglionic and postganglionic sympathetic fibers. In either case, sensitization attains a maximum within two weeks after denervation.

Inferences have been drawn from the available data concerning the nature of sensitization (Cannon and Rosenblueth, 1937), but no direct evidence has been presented. Whether sensitization after denervation is a permanent change in the state of the effector or persists for only as long as nerve impulses fail to reach it has not been studied. That problem forms the subject of this investigation because of the interest primarily inherent in it and because of the information it may give concerning the nature of the sensitization of structures by denervation.

METHOD. Adult cats were used throughout these experiments. The nictitating membrane was chosen as the effector for study, because it is favorable for solution of the problem and has already been the subject of numerous other related investigations. The membrane was denervated aseptically by interrupting the cervical sympathetic proximal or distal to the superior cervical ganglion. Preganglionic denervation was done in eight cats; postganglionic in five. The preganglionic fibers were cut (5 cases) or crushed (3 cases) 1.0 to 1.5 cm. proximal to the superior cervical ganglion. No sutures were used to approximate the severed nerve ends. Care was taken, however, to dissect free of its sheath only a very small stretch of nerve (1.5 mm.) so that after severing the trunk the cut ends remained in close approximation. A similar technique was used when the

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fibers were crushed. Of the five cases in which postganglionic regeneration was studied, the fibers were cut in four animals and crushed in one. In five cats, preganglionic and postganglionic fiber regeneration was studied on alternate sides of the same animal. In four animals, the membrane was denervated on only one side, the other serving as normal control. In cat 1, the postganglionic fibers were crushed on one side and the superior cervical ganglion was excised from the other side to make regeneration there impossible (Langley, 1900).

Simultaneous kymographic records were made of the responses of the right and left nictitating membranes to graded doses of adrenalin injected intravenously (0.25, 0.50, 0.75, and 1.00 cc. of 1:100,000 adrenalin). The dilution was freshly prepared for each experiment from a standard solution (Parke-Davis). All dilutions were made with mammalian Ringer solution and each dose was made up to 1.0 cc. before injection. The solution was injected at a uniform rate of 1.0 cc. in about 10 seconds. The first records were taken either immediately before denervation or immediately afterward, and the determinations were then repeated at about weekly intervals. The recording levers were adjusted to exert equal tension on the two sides and to afford a 19-fold magnification with a minimum of friction. The small scar on the membrane left by the first application of the serrefine to its margin made it possible to record from the same point on the membrane each time. As soon as a definite change in sensitivity was ascertained, regeneration was suspected and tested by faradic stimulation of the cervical sympathetic with pupillodilatation and retraction of the nictitating membrane as indicators.

For records and operations nembutal anesthesia (0.7 to 0.8 mgm. per kgm. injected intraperitoneally) was used. Only animals in which all observations were carried to completion are included in this report.

Results. 1. *Reaction of the nictitating membrane to adrenalin during degeneration and regeneration of its nerve supply.* Figure 1 shows the reactions of the normal nictitating membrane of the cat to adrenalin injected intravenously. A few animals gave no response at all to the smallest dose used (0.25 cc. of 1:100,000), but, in general, the variation between animals was only slight. An exception was found in cat 8, not included elsewhere in this report, in which the nictitating membranes repeatedly gave relatively large responses to adrenalin (fig. 2) and reacted generally as if they had been sensitized by denervation or cocaine.

Figure 6 represents the reactions to adrenalin of right and left nictitating membranes after development of maximal sensitivity, the former by postganglionic and the latter by preganglionic denervation two weeks previously. These observations confirm those of Hampel (1935), who found that the development of sensitivity reached a maximum within 15 days of denervation whether preganglionic or postganglionic. Figure 7 shows

the reactions of the same membranes as in figures 1 and 6, but 32 days after the denervation. The sensitivity of the right membrane remains about the same, but that of the left membrane (preganglionic denervation) has decreased. The record in figure 3 was made 16 days later and shows practically complete return of the left membrane to normal sensitivity and partial loss of sensitivity in the right membrane. Figure 4 represents the responses of the same membranes 73 days after denervation. They both now give nearly the same responses to adrenalin as before denervation (fig. 4A). A comparison of the response of the right membrane to a slightly larger dose of adrenalin (0.75 cc. of 1:100,000) with that of the left membrane to the same dose, however, shows that the former still

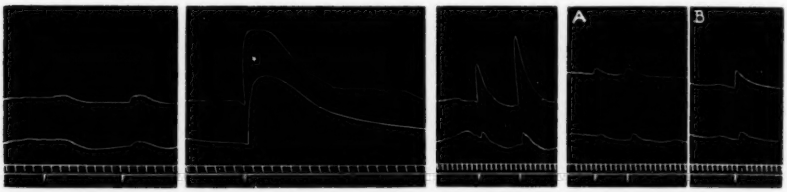


Fig. 1 Fig. 2 Fig. 3 Fig. 4

Fig. 1. Cat 1. Nembutal (0.7 cc. per kgm. intraperitoneally). Upper record, right, and lower record, left nictitating membrane. Both membranes with intact nerve supply. First signal: 0.25 cc. adrenalin, 1:100,000 injected intravenously; second signal: adrenalin, 0.5 cc. Both doses made up to 1.0 cc. before injection. Time: 30 seconds.

Fig. 2. Cat 8. Nembutal (0.7 cc. per kgm. intraperitoneally). Upper record, right, and lower record, left nictitating membrane. Both membranes with intact nerve supply. Signal: 0.25 cc. adrenalin, 1:100,000, diluted to 1.0 cc., was injected intravenously. Time: 30 seconds.

Fig. 3. As in figure 7, but 16 days later still.

Fig. 4A. As in figures 1, 3, 6 and 7, but 73 days after the denervations.

B. Shows the response to 0.75 cc. adrenalin, 1:100,000, diluted to 1.0 cc. The right membrane is still somewhat more sensitive than the left.

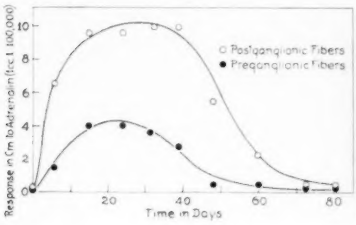
possesses a slightly increased sensitivity (fig. 4B). Records made $4\frac{1}{2}$ months after denervation showed no further change.

In all cases, faradic stimulation of the cervical sympathetic revealed functional continuity as soon as a definite decrease in sensitivity to adrenalin had occurred. It may be stated, then, that the increased sensitivity of the nictitating membrane to adrenalin is lost with regeneration of the sympathetic nerve fibers. Conversely, it is proper to consider the first definite decrease in sensitivity as an indication of the reëstablishment of functional continuity for at least some of the nerve fibers.

2. *The "paradoxical pupillary reaction."* Among the effects of interrupting the cervical sympathetic nerve trunk the most characteristic is

the development of "Horner's syndrome"—i.e., myosis, enophthalmos, narrowing of the palpebral fissure, and, in some animals (e.g. the cat and dog), spread of the nictitating membrane over the inner half of the eyeball. In two of the nine animals in this report, the signs were not fully developed until the second postoperative day. In all cases the paralysis was more marked after postganglionic than after preganglionic denervation, suggesting that the superior cervical ganglion was still capable of influencing its effectors autonomously after decentralization (cf. Govaerts, 1936, for the stellate ganglion).

In an animal with a well-developed "Horner's syndrome" unilaterally, slight excitement, or exposure to cold, or asphyxia leads to pupillary dilatation and retraction of the nictitating membrane. The widening of the pupil exceeds that in the intact control side, which ordinarily presents



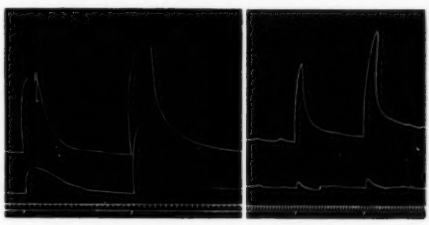


Fig. 5 Fig. 6 Fig. 7

Fig. 5. The responses of the nictitating membranes of cat 2 to 0.25 cc. adrenalin, 1:100,000, at intervals after postganglionic denervation of the right nictitating membrane and preganglionic of the left. Ordinates: responses in cm. x 19. Abscissae: days after denervation.

Fig. 6. Same as in figure 1, but 2 weeks after sectioning postganglionic fibers to the right membrane and preganglionic fibers to the left.

Fig. 7. As in figure 6, but 32 days after the denervation.

a wider pupil than the denervated side. This phenomenon, the "paradoxical pupil," was attributed by Elliott (1905) to increased sensitivity to adrenine. The paradoxical pupillary reaction was not seen in the cats used in these experiments unless they were excited or exposed to cold, conditions in which the adrenal glands are activated (Cannon, 1929).

In seven of the nine animals studied, regeneration of nerve fibers was accompanied by enlargement of the ipsilateral pupil beyond normal size. The corresponding nictitating membrane was likewise more strongly retracted. This "paradoxical" effect after regeneration of preganglionic and postganglionic fibers had already been observed by Machida (1929) and Lee (1930). The phenomenon was especially convincing in the four animals in which denervation was unilateral, the other side serving as normal control. In cat 9, in which the left superior cervical ganglion had been

excised, regeneration of the right postganglionic fibers caused a nearly maximal widening of the corresponding pupil, strong retraction of the nictitating membrane, and marked exophthalmos. The animal showed obvious impairment of its visual mechanism, probably a disturbance of accommodation to near objects. The condition continued unchanged for 10 days and then slowly became less and less pronounced until, 39 days later, the eye looked normal.

The over-enlargement of the pupil occurred, for both preganglionic and postganglionic regeneration, approximately one week after the first evidence of decreasing sensitivity to adrenalin, i.e., one week after functional regrowth of nerve fibers had started. It persisted longer for postganglionic regeneration than for preganglionic, an average of 31 days (maximum 39; minimum 26 days) and 22 days (maximum 38; minimum 2 days) respectively. In either case, some increased sensitivity to adrenalin lasted at least one week after the over-enlarged pupil had returned to normal size.

3. *The time required for loss of sensitivity to adrenalin associated with regeneration of pre- and postganglionic sympathetic nerve fibers.* The average length of time elapsed between the interruption of the preganglionic nerve fibers and the first definite decrease in sensitivity of the nictitating membrane to adrenalin was 3 weeks. It was slightly less ($2\frac{1}{2}$ weeks) for cases in which the fibers were crushed instead of being cut (cf. Lee, 1929). The shortest time was 2 weeks; the longest $4\frac{1}{2}$ weeks. In the postganglionic group, the average time required was 7 weeks. The shortest time was for the one animal in this group in which the postganglionic fibers were crushed instead of cut, $5\frac{1}{2}$ weeks. The longest time required was $8\frac{1}{2}$ weeks.

In general, disappearance of the increased sensitivity to adrenalin was more rapid with preganglionic than with postganglionic regeneration, 2 weeks and 7 weeks (average) respectively (fig. 5).

On the assumption that the loss of increased sensitivity of the nictitating membrane begins with the first functional regrowth of the preganglionic and postganglionic fibers, a table is presented below showing the time required for the most rapidly regenerating nerve fibers to establish functional continuity in the cervical sympathetic of the cat. It should be noted that the preganglionic fibers had to regrow for a distance of only about 1.5 cm., while the postganglionic fibers had to regenerate for a distance of 5 or 6 cm.

Discussion. Studies on the regeneration of sympathetic nerve fibers have been relatively few, particularly studies on the regeneration of postganglionic fibers. The available data indicate that 3 to 10 weeks are required for the regeneration of preganglionic fibers (see Lee, 1929, for references). Langley (1897) observed that the paralytic symptoms from cutting the cervical sympathetic of the cat begin to disappear as soon as

8 to 12 days after section, but confirmation of regeneration by electrical stimulation was not obtained until after 24 days. The time required for the regeneration of postganglionic fibers in the cervical sympathetic has been 7 to 11 weeks (Langley, 1897; Machida, 1929). Tower and Richter (1932) found no evidence of regeneration of the postganglionic fibers from the stellate ganglion of the cat even 12 to 18 months after severing them, which they attributed to possible injury to the ganglion.

In the experiments reported above, the earliest evidence of regeneration (decreased sensitivity to adrenalin) came 15 days after preganglionic section and was confirmed 3 days later by electrical stimulation. For postganglionic fibers, the earliest signs of regeneration came $5\frac{1}{2}$ weeks after interruption of the nerve fibers, confirmed one week later by electrical stimulation of the preganglionic fibers. Regeneration took place more rapidly after crushing the nerve fibers than after cutting them (table 1). The number of experiments, however, was small and it is impossible to

TABLE 1

Time required for regeneration of preganglionic and postganglionic fibers in the cervical sympathetic of the cat

	PREGANGLIONIC			POSTGANGLIONIC		
	Minimum	Maximum	Average	Minimum	Maximum	Average
	weeks	*weeks*	*weeks*	*weeks*	*weeks*	*weeks*
Cut	3	$4\frac{1}{2}$	$3\frac{1}{2}$	7	$8\frac{1}{2}$	$7\frac{1}{2}$
Crushed	2	3	$2\frac{1}{2}$	$5\frac{1}{2}$	*	*
Total	2	$4\frac{1}{2}$	3	$5\frac{1}{2}$	$8\frac{1}{2}$	7

* Only one animal.

draw general conclusions from them. It is difficult to understand why severed but well-approximated nerve fibers should regenerate more slowly than fibers interrupted by crushing. For preganglionic nerves Langley (1897) and Tower and Richter (1931) found that the distance the fibers had to regrow exerted relatively little influence on the total time required for functional regeneration. The latter authors have made the interesting suggestion that perhaps a considerable length of time elapses in the establishment of functional connections at the synapse.

The evidence presented here indicates that the increased sensitivity to adrenalin of the denervated nictitating membrane of the cat is not an irreversible change but is lost when the nerves have regenerated. Regenerating nerves are said to attain functional continuity some time before they respond to direct electrical stimulation (Howell and Huber, 1892). Since it is sometimes difficult to be sure that the stimulating electrodes are not on newly regenerated fibers, the decrease in the sensitivity to adrenalin

acquired by denervation may serve as a better index for regeneration than faradization of the nerve trunk.

The question arises, is extraordinary sensitivity to chemical and other stimuli an artificial phenomenon occurring under the influence of drugs or produced by interfering with the sympathetic nerve supply, or may it occur without drugs and without interfering with the nerve supply? One animal (8) has been mentioned above in which the membranes responded consistently as if they had been sensitized by denervation or by the injection of cocaine (fig. 2). It seems, then, that subjects may be found, though rarely, in which smooth muscle is unusually sensitive. Indeed, Heinbecker and Bishop (1934) and Lewis (1936) have suggested that Raynaud's disease is due to an increased sensitivity of local peripheral vessels rather than to a general hyperactivity of the sympathetic nervous system.

The overcorrection of Horner's syndrome in animals during regeneration of the cervical sympathetic was observed by Machida (1929) and Lee (1930). No adequate explanation has been offered, although Machida (*loc. cit.*) has suggested that possibly some of the pilomotor fibers may regrow as pupillodilators (Langley, 1897), so that this organ may then receive more than its quota of nerve impulses. This, however, would not explain the return of the pupil to normal as the regeneration progressed. It should be emphasized, moreover, that not only is the pupil wider than normal, but the nictitating membrane is more strongly retracted and exophthalmos may be marked. Increased sensitivity to circulating hormones does not account for the phenomenon. In cat 9, when the paradoxical reaction was greatest, the corresponding nictitating membrane was less sensitive to adrenalin than the contralateral one. Furthermore, the phenomenon always appeared at the same time that the sensitivity to adrenalin was decreasing. The situation might be easily explained by assuming that with regeneration the nerve fibers find a sensitized effector which gives an abnormally large response to the normal impulses (sympathin) delivered to it. As regeneration proceeds, however, the increased sensitivity decreases and the phenomenon therefore is gradually lost.

It is of interest that the "paradoxical pupillary reaction" and the signs associated with it disappeared more rapidly with preganglionic than with postganglionic regeneration. Likewise, the increased sensitivity of the nictitating membrane to adrenalin fell more rapidly with preganglionic than with postganglionic regeneration. Indeed, complete loss of the increased sensitivity after postganglionic denervation has not been attained even after allowing $4\frac{1}{2}$ months for regeneration. The difference probably reflects the difference in innervation of ganglion and nictitating membrane. In the ganglion, although the ratio of postganglionic to preganglionic neurones is commonly taken to be 32:1, there is considerable overlap in

the innervation of the postganglionic neurones by preganglionic fibers (cf. Eccles, 1935). The complete functional innervation of the ganglion cells, therefore, would not necessarily depend on complete anatomical regeneration of the preganglionic fibers. In the nictitating membrane, however, there is probably no such overlap. In fact, according to the histological evidence (see Cannon and Rosenblueth, 1937, for references), only some of the smooth-muscle cells may be innervated. Failure of a postganglionic fiber to regrow, then, would leave at least one and perhaps more cells denervated, and increased sensitivity would persist. The fact that, though not complete, the loss of sensitivity with postganglionic regeneration has reached a steady level after 4 months suggests that perhaps regeneration of all the postganglionic fibers is not to be expected.

The theories concerning the nature of the increased sensitivity of smooth muscle to adrenalin after denervation have been summarized by Hampel (1935) and Rosenblueth and Cannon (1936). The idea has been favored that increased sensitivity to sympathin and adrenalin may at least in part be due to an increased permeability of the effector cell membranes. More recently, Baeq (1936) has suggested that denervation renders smooth muscle more capable of "fixing" phenolic compounds within its cells. Hence the denervated nictitating membrane is sensitized not only to adrenalin but also to the action of the anti-oxydant pyrogallol. The nature of the change which makes denervated cells better able to fix phenolic amines, however, remains the problem. The change might still be fundamentally one of altered permeability of cell membranes, or the tissue might be made less capable of destroying compounds of the phenolic group. In the absence of more data, discrimination between the different possibilities would be purely speculative.

Whatever may be the nature of sensitization by denervation, studies of the effects of degeneration and regeneration of nerve supply have made possible some general comments. In the first place, the development of sensitivity after denervation is a gradual process, increasing rapidly at first and then more slowly until a maximum is reached after about two weeks. This would suggest that the development of sensitivity might possibly depend upon a structural degeneration of the axons for which two weeks might be required. Sensitization, however, can be brought about by preganglionic denervation, the postganglionic fibers remaining intact. A closer relationship appears to exist between the development of sensitivity and the presence or absence of nerve impulses to the effector in question. Evidence has been presented (see p. 469) that the superior cervical ganglion may send impulses to its effectors autonomously after preganglionic denervation, but, of course, with diminished intensity. Impulses are excluded entirely when the postganglionic fibers are interrupted. Correspondingly, the sensitization following preganglionic de-

nervation is always less than that following postganglionic denervation. The functional change following the exclusion of impulses from the smooth-muscle cells develops gradually and reaches a maximum after 2 weeks. Conclusions regarding the nature of the change, however, must await further studies.

SUMMARY

The sensitivity of the cat's nictitating membrane to adrenine was studied during degeneration and regeneration of its preganglionic or postganglionic nerve supply. The increased sensitivity was greater after postganglionic than preganglionic denervation. In either case, however, it reached a maximal level after about 2 weeks (figs. 5 and 6).

With regeneration of the nerve supply the increased sensitivity began to decline; the period of the decline to normal for preganglionic and to nearly normal for postganglionic fibers averaged 2 and 7 weeks respectively (see e.g., fig. 5).

The average length of time for the regeneration of the faster fibers in preganglionic nerves was 3 weeks and for postganglionic fibers 7 weeks. Regeneration was somewhat faster after crushing than after cutting the nerves (table 1).

The bearing of these findings on the problem of the nature of the sensitization of smooth muscle by denervation is discussed (p. 473).

It is a pleasure to thank Dr. W. B. Cannon and Dr. A. Rosenblueth for their helpful suggestions during the course of this work.

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THE EFFECT OF TEMPERATURE ON THE VOLUME FLOW OF BLOOD THROUGH THE SYMPATHECTOMIZED PAW OF THE DOG WITH OBSERVATIONS ON THE OXYGEN CONTENT AND CAPACITY, CARBON-DIOXIDE CONTENT, AND PH OF THE ARTERIAL AND VENOUS BLOOD

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Previous studies on the effect of temperature on the rate of blood flow in the normal and in the sympathectomized hand (1) suggested a dual control of the circulation. Through the vasomotor nerves, the flow of blood is considered to be modified in accordance with the requirements for thermo-regulation of the body as a whole. After removal of the vasomotor control, the circulation is apparently dependent upon the metabolic requirements of the tissues. In order to investigate the relationship between blood flow and tissue metabolism, determinations of the oxygen utilization and carbon-dioxide production were necessary. The volume flow of blood through the sympathectomized hand was therefore measured and samples of blood were taken from veins on the dorsum of the wrist. The oxygen and carbon-dioxide contents of the venous blood, taken with the hand at different temperatures, were determined, but wide variations were encountered. Since the taking of the blood samples was accompanied by unavoidable emotional disturbances, and since it has been shown that adrenal secretion reduces the peripheral blood flow in man (2), it was thought that circulating adrenine might have reduced the flow of blood at the time of the venepuncture and, in this way, have produced the discordant results. It seemed essential to conduct studies on animals in which the reflex secretion of adrenine could be excluded. In these experiments the effect of changes in temperature on the volume flow of blood was accordingly studied in the sympathectomized paw of the dog. The adrenal factor was eliminated by removal of one gland and denervation of the other. The venous blood coming from the paw was compared with the arterial blood with respect to oxygen content and capacity, carbon-dioxide content, and hydrogen ion concentration.

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[2] Assistant Resident in Medicine.

METHOD. Three female hounds were studied, S, 3 years old; M, 8 years old; and C, 5 years old. Their weights were 20, 18, and 22 kilos respectively. In dogs C and S, one adrenal gland and both lumbar sympathetic chains (from L 1 to L 5 inclusive) were removed, and the major and minor splanchnic nerves were cut on the side on which the adrenal gland was left intact. All operations were performed under ether anesthesia. In dog M, in addition to the ganglionectomy and adrenal operation, the right sciatic nerve was crushed at a second operation. In this dog anesthesia of the paw extended to the ankle. The completeness of the sympathectomy and exclusion of adrenine was confirmed by the absence of any decrease in the volume flow of blood through the paw when the animal was stimulated, although an increase in the pulse rate and dilatation of the pupil occurred under such circumstances, indicating that the animal had reacted to the stimulation.

The volume flow of blood was measured by a previously-described modification (1) of the plethysmographic technique of Hewlett and Van Zwaluwenburg. Instead of a Brodie's bellows to measure the increase in volume, a miniature Krogh spirometer was used. The reservoir measured 4 x 3 x 3 cm. and was made of brass sheeting 1/64 in. in thickness. The float was made of brass foil 0.002 in. in thickness. The top surface measured 3 x 2 cm. The ends were formed of segments of a cylinder of 3 cm. radius. One end of the float rested by a hole and groove upon two adjustable screw points. Maximum displacement through an arc of 36 degrees was obtained for an increase in volume of 6 cc. The reservoir was filled with mineral oil to avoid the effects of surface tension, and the float was counterbalanced with an adjustable weight. The rise of the spirometer float was recorded by a heart lever which had its axis 7 cm. behind the free edge of the float. By setting back the axis, forward and backward displacement at the writing point was decreased. One centimeter elevation of the writing point on the kymograph indicated approximately 1 cc. increase of volume in the plethysmograph.

The dogs had fasted for 18 hours before each experiment. They were trained to lie quietly on their sides, and the upper hind paw was inserted into the plethysmograph. The hair above the ankle was first clipped with scissors. Particular care was taken not to cut the skin nor to pull the hairs because of the possibilities of producing a traumatic hyperemia. Rubber cement was then applied over a band 1 in. wide and a fitted rubber cuff, similar to the one previously described (1), was applied with the collar extending towards the body. Because of the presence of hair it was difficult to avoid leakage. With an adequate quantity of rubber cement, any small leak was controlled by direct pressure applied for a short time.

The skin above the ankle of the dog is loose, and the tissues are stretched out by the tendon of the gastrocnemius. Compression of the veins by the cuff itself is impossible if the cuff is carefully fitted to the skin. Since the inflow of blood would be decreased by engorgement of the paw, the importance of a properly fitted cuff is evident.

The volume of the paw was determined after each experiment, and was found to range between 170 and 210 cc. It was measured by the displacement of water from a cylinder.

Ten cubic centimeters of water were removed from the plethysmograph to allow for air transmission of changes in volume to the spirometer. To calibrate the spirometer, a 10 cc. oiled syringe was used so that the volume of air should be the same as that present in the plethysmograph during the experiments. With a counterpoised heart lever and float, the measured difference in excursion between the first and second cubic centimeter quantities of air forced from the syringe to the spirometer was between 5 and 10 per cent. No difference was observed between the second and third cubic centimeter of displacement.

A pneumatic cuff was wrapped about the leg just above the ankle so that it overlapped the edge of the fitted rubber collar. The cuff was connected to a liter pressure bottle equipped with a mercury manometer. By means of a valve, any desired pressure could be applied to the tissues of the leg above the ankle. The cuff was constructed of "dental rubber dam." It measured 12 x 3 cm. and was enclosed in a cloth bag 22 x 3 cm.

The systolic blood pressure was measured by determination of the pressure, applied through the cuff, which was just sufficient to prevent the flow of blood into the paw. This determination was repeatedly checked by palpation of the tibial artery just distal to the cuff. The pressure necessary to prevent inflow of blood was 5 to 10 cm. higher than that necessary to prevent palpable pulsation. The diastolic blood pressure was measured by determination of the pressure which was just sufficient to prevent the inflow of blood during diastole. This pressure could only be measured at the more rapid rates of flow, i.e., when the inflow of arterial blood continued throughout diastole. The systolic blood pressures varied between 115 and 155 (table 1), and the diastolic pressures between 80 and 110 mm. of mercury.

The optimum collecting pressure to prevent venous outflow, and, at the same time, not to hinder arterial inflow, was found to be 80 mm. of mercury. In the experiments this pressure was applied until the rate of increase in paw volume started to decline. The pressure was then released until the volume of the paw had reached the base line. At least 5 determinations of the volume flow of blood were made at each temperature, and the mean was selected.

In a former communication (1) the time interval for stabilization of blood flow at any temperature was given as 5 to 15 minutes. In the present experiments the temperature at the depth of 2.5 cm. inside the paw was measured in relation to the blood flow and bath temperature. One of the dogs (M), had an anesthetic paw, and another (S), was anesthetized with Nembutal. Between 30 and 45 minutes were required for a stable temperature to be reached inside the paw. In all of these experiments, at least 60 minutes were allowed at low temperatures to ensure stability before blood flow readings were taken, and at least 45 minutes were allowed at the higher temperatures.

At low temperatures, with a slow rate of blood flow, 2.0 cc. per minute, the internal temperature, 16.6°C., was close to that of the bath, 16.0°C. At intermediate temperatures with a more rapid rate of flow, 4 cc. per minute, the internal temperature, 30.2°C., varied considerably from that of the bath, 25.7°C. Again at high temperatures, the internal temperature, 36.0°C., approached that of the bath, 34.8°C. The difference in temperature between the surface and the inside of the paw, which varied not only with the temperature of the bath but also with the volume flow of blood, made it impossible, at any specific bath temperature, to be certain of the temperature of each part of the paw. Since the discrepancies between external and internal temperature were less at low temperatures, and again, at temperatures close to that of the body, these extremes were chosen more frequently than the intermediate temperatures (see fig. 4).

In the earlier experiments the paw was immersed in water below 10°C. and the temperature of the bath allowed to rise. Certain variations in blood flow and oxygen content of the venous blood led us to suspect the possibility of trauma from cold. Therefore in subsequent experiments, immersion in water below 14°C. was avoided. The bath temperature was reduced to 14°C. and kept near this level by adding ice to the water in the plethysmograph.

Although vasomotor effects from exposure to cold were not observed in the sympathectomized paw without adrenal secretion, the effect on the systemic blood pressure of cold applied to the paw was definitely noticeable in several experiments. In an

TABLE 1

The effect of temperature on the blood flow and metabolism of the paw; in dogs S, M, and C the paws were sympathectomized and the adrenals inactivated; in dog M, the paw was also totally denervated

The data from 19 additional experiments have been omitted from this table for purposes of brevity.

EXPERIMENT NO.	DOG	DATE	BATH TEMPERATURE	BLOOD FLOW, CUBIC CENTIMETER PER 100 CC. PAW VOLUME PER MINUTE	ARTERIAL O_2		VENOUS O_2		ARTERIAL-VENOUS OXYGEN DIFFERENCE	O_2 UTILIZATION, CUBIC CENTIMETER PER 100 CC. PAW VOLUME PER MINUTE	CO_2 CONTENT		ARTERIAL-VENOUS CO_2 DIFFERENCE	pH		BLOOD PRESSURE
					Content	Capacity	Content	Capacity			Arterial	Venous		Arterial	Venous	
		1936	*°C.*		*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*		*vol. per cent*	*vol. per cent*	*vol. per cent*			*mm. Hg*
1	S	7/27	15.5	3.5	16.24	17.66	14.59	17.53	1.65	0.057	44.36	43.16	−1.20	7.42	7.37	130
			35.2	10.2	16.81	18.26	15.74	17.24	1.07	0.112	40.50	44.36	3.86	7.42	7.37	140
2	S	7/22	14.4	5.4	16.76	18.03	16.50	17.84	0.26	0.014	39.05	42.70	3.65	7.42	7.41	150
			33.9	11.8	16.53	17.68	15.53	17.62	1.00	0.118	38.70	41.85	3.15	7.41	7.40	135
3	S	7/21	14.8	5.4	16.70	17.90	16.43	17.85	0.27	0.014	40.12	41.95	1.83			145
			33.6	17.5	16.47	17.40	15.61	17.43	0.86	0.150	39.85	41.42	1.57			155
4	S	7/20	17.0	5.9	16.78	18.50	16.38	17.82	0.40	0.024	38.90	42.23	3.33			
			36.9	7.5	16.98	17.75	15.03	17.53	1.95	0.146	39.95	42.70	2.75			145
5	S	7/17	17.0	4.1	17.00	18.12	16.07	17.67	0.93	0.038	39.40	41.80	2.40			
			35.3	9.7	16.68	18.52	16.00	18.12	0.68	0.066	39.70	41.30	1.60			150
6	M	7/16	20.8	1.7	13.08	14.58					42.55			7.39		150
			34.6	14.2	13.75	15.24	12.20	15.84	1.55	0.220	42.72	44.45	1.73	7.39	7.36	135
7	M	7/14	20.0	2.6	13.19	13.94	11.20	13.88	1.99	0.052	41.62	41.72	0.10			
			35.4	17.2	13.30	13.94	10.86	13.82	2.44	0.423	40.83	41.62	0.79			125
8	M	7/13	18.5	1.6	13.36	14.55	10.98	14.56	2.38	0.038	40.90	41.60	0.70			
			34.4	14.3	14.23	15.63	12.57	15.75	1.66	0.237	40.03	41.35	1.32			125
9	M	6/8	17.3	3.2	14.43		12.74	15.03	1.69	0.054	41.15	41.65	0.50			140
			35.2	15.6	14.85	16.70	13.28		1.57	0.245	40.30	42.03	1.73			120
		(After stimulation)			(15.50)	(16.47)	(13.28)		(2.22)	(0.346)	(39.35)	(42.03)	(2.68)			
10	M	6/5	20.6	6.2	15.80	17.20	14.45	17.33	1.35	0.083	42.60	42.60	0.00			
			29.3	11.1	14.20		14.00		0.20	0.022	40.85	42.60	1.75			125
			36.3	19.4	15.35		14.25		0.10	0.019	41.77	42.90	1.13			115
11	C	7/29	14.3	4.2	18.42	19.30	17.85	19.30	0.57	0.024	34.73	35.82	1.09	7.41	7.38	135
			21.8	10.6	18.26	19.30	17.35	19.18	0.91	0.096	35.20	36.00	0.80	7.40	7.37	130
			32.5	20.0	18.14	19.30	17.60	19.47	0.54	0.108	35.52	36.33	0.81	7.41	7.38	145
12	C	7/23	14.6	5.9	18.74	19.80	17.46	19.95	1.28	0.075	36.37	37.10	0.73	7.43	7.41	145
			35.1	16.9	18.63	19.72	18.12	19.80	0.51	0.086	36.75	38.13	1.38	7.43	7.43	145
13	C	6/23	15.0	2.1	21.05		19.00	21.00	2.05	0.043	35.93	38.30	2.37	7.41	7.37	138
			28.0	8.8			19.75					37.80			7.36	
			36.7	20.0	19.90	21.00	19.83		0.07	0.014	35.85	37.62	1.77	7.39	7.37	
14	C	6/22	15.0	1.5	19.96	22.05	17.60		2.36	0.035	36.55	40.28	3.73			132
			29.2	6.0	20.40		19.70		0.70	0.042	37.08	37.95	0.87			132
			39.2	10.2	19.92	23.90	19.63		0.29	0.029	37.22	36.83	0.39			140
15	C	6/19	16.3	2.2	20.25		18.65	21.15	1.60	0.035	39.53	42.57	3.04	7.43	7.40	
			26.0	6.8			18.75					40.10			7.40	122
			35.0	13.6	20.95	21.90	18.85		2.10	0.285	36.25	39.20	2.95	7.43	7.40	132

effort to offset this influence, a heating pad was used, and the rectal temperature maintained close to 38.4°C.

Blood for analysis was obtained from the lesser saphenous vein as it courses posteriorly just above the ankle. A no. 20 gauge needle was used which was sharpened before each puncture. Stasis was entirely avoided. Venepuncture was performed without any recognizable disturbance of the animal. Samples of arterial blood were obtained with a no. 20 gauge needle from the femoral artery, without removing the contralateral paw from the plethysmograph. Although precautions were taken not to excite the dog, it was difficult to secure the arterial blood without some disturbance. Consequently, the venous sample was always taken just before the arterial sample. In most instances the samples were taken within 5 minutes of each other.

Eight cubic centimeters of blood were drawn into a syringe oiled with neutral paraffin oil (White, Oil Petrolatum, Central Scientific Co., Boston) which had previously been saturated with a mixture of 5 per cent carbon-dioxide and 95 per cent oxygen. The blood was transferred anaerobically to tubes containing a similar oil. In the bottom of the tubes, 0.2 cc. of a 10 per cent potassium oxalate solution of pH 7.40 had been evaporated in a stream of air (3). In earlier experiments heparin was used as an anti-coagulant, but was replaced by the oxalate when the pH was determined.

The blood was stirred with a footed glass rod and immediately chilled by immersion in ice water. It was kept at 4°C. until the gas determinations were made.

The oxygen content and capacity, and the carbon-dioxide content of the arterial and venous bloods were determined in duplicate by the method of Van Slyke and Neill (4). The pH was determined by the method of Shock and Hastings (5).

Results. As the temperature of the bath was increased, there followed a rise in the rate of blood flow in the paw which is illustrated in figure 1 (taken from expt. 11, table 1). In cold water, 14.3°C., the volume flow of blood was 4.2 cc. per 100 cc. paw volume per minute. At 32.5°C. the blood flow had increased to 20.0 cc. per minute. Analyses of the arterial and venous bloods showed that the oxygen and carbon-dioxide contents and pH were constant, within experimental limits, at each of the three temperatures. Essentially similar data from both dogs with sympathectomized paws and the dog with the totally denervated paw are summarized in table 1.

Figure 2 represents the effect of changes in bath temperature on the volume flow of blood, in all the experiments. Although, from day to day, wide variations were encountered, the slope of the curve for any one experiment was quite constant.

No correlation was noted between the difference in oxygen contents of the arterial and venous bloods and either the blood flow or the temperature of the bath. Figure 3 illustrates this lack of correlation between arterio-venous oxygen difference and blood flow. Fifty-nine determinations of the arterio-venous oxygen difference ranged between 0.07 and 2.83 volumes per cent. With an average arterial oxygen saturation of 93 per cent, the venous oxygen saturation averaged 86.9 per cent.

The difference in carbon-dioxide content of the arterial and venous

blood was also not related to the blood flow or bath temperature. Figure 4 shows the absence of relationship between arterio-venous carbon-dioxide difference and bath temperature. A chart similar to figure 3 would result if the arterio-venous carbon-dioxide difference were plotted against blood flow.

Since the circulation through the sympathectomized paw varies directly with the temperature of the bath, and since the differences in oxygen and carbon-dioxide contents of the arterial and venous bloods are independent of the bath temperature or flow of blood, it is to be expected that the oxy-

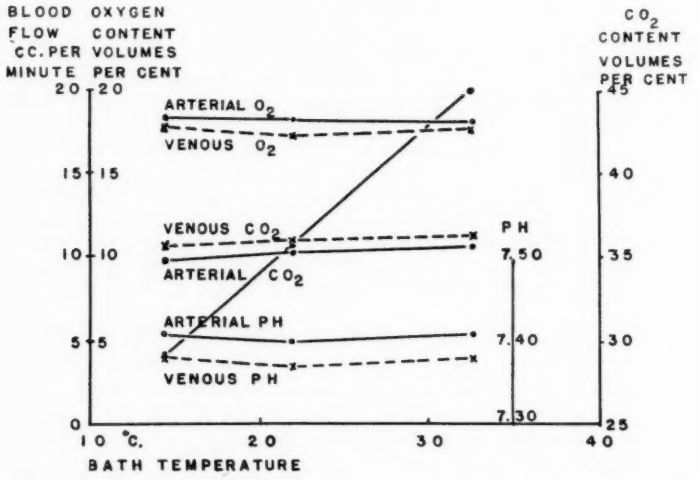

Fig. 1. The effect of changes in local temperature on the volume flow of blood through the paw; oxygen, and carbon-dioxide contents and pH of the arterial and venous bloods. Dog C, experiment 11, table 1. Ordinates, blood flow in cubic centimeters per minute per 100 cc. paw volume; oxygen and CO_2 contents in volumes per cent; pH. Abscissae, temperature of bath in degrees Centigrade.

gen utilization and carbon-dioxide production should vary directly with the bath temperature. Figure 5 demonstrates the effect of changes in the temperature of the bath on the oxygen utilization of the paw. A graph which shows a similar relationship between carbon-dioxide production and temperature has not been included.

The pH of the arterial and venous bloods remained constant during any one experiment, within the limits of error of the method. The variations in pH were too small and the limits of error too great to permit any conclusions to be drawn concerning the relationship between pH and arterio-venous oxygen difference.

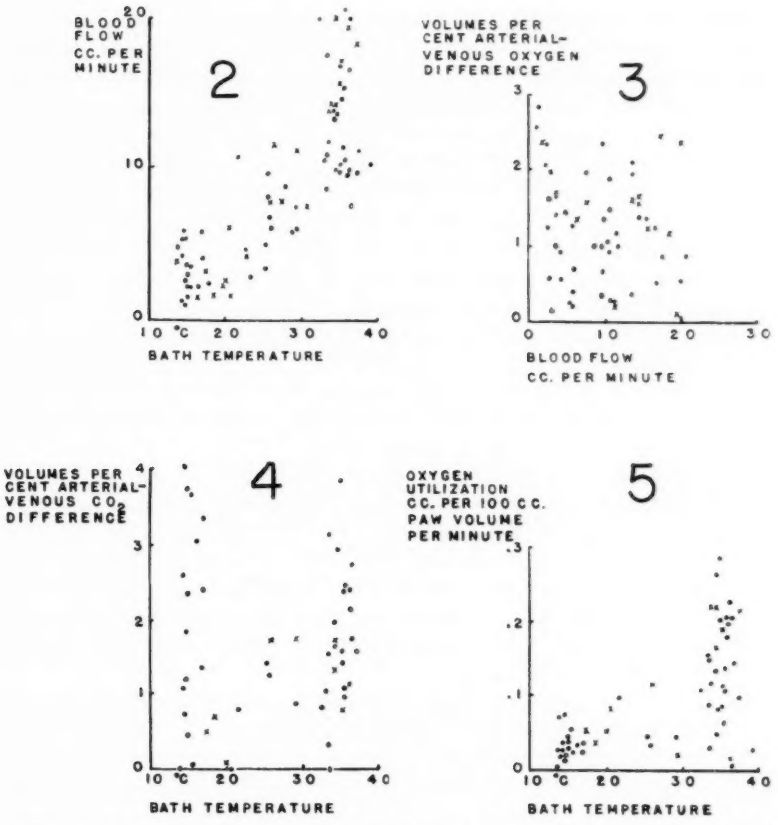

Fig. 2. The effect of changes in local temperature on the volume flow of blood through the paw: composite chart from all the experiments. Ordinates, blood flow in cubic centimeters per minute per 100 cc. paw volume. Abscissae, temperature of bath in degrees Centigrade. In this, as in the following figures, dots and circles indicate the values obtained in the sympathectomized paws of dogs S and C: crosses, the results found in the totally denervated paw of dog M.

Fig. 3. Effect of variations in blood flow on the arterio-venous oxygen difference. Composite chart from all the experiments. Ordinates, arterio-venous oxygen difference in volumes per cent. Abscissae, blood flow in cubic centimeters per minute per 100 cc. paw volume.

Fig. 4. Effect of variations in local temperature on the arterio-venous carbon-dioxide difference. Composite chart from all the experiments. Ordinates, arterio-venous carbon-dioxide difference in volumes per cent. Abscissae, bath temperature in degrees Centigrade. Three negative values have not been included.

Fig. 5. Effect of variations in local temperature upon the oxygen utilization of the paw. Composite chart from all the experiments. Ordinates, oxygen utilization in cubic centimeters of oxygen per 100 cc. paw volume per minute. Abscissae, bath temperature in degrees Centigrade.

Discussion. The accuracy of the plethysmographic method has not been compared with that of other methods of measuring the blood flow through the extremities. In isolated organs, however, Brodie, according to Hewlett and Van Zwaluwenburg (6), found that the results obtained by this method agreed closely with those obtained with Ludwig's stromuhr. The range of blood flows in our experiments is in general agreement with that reported by Tschuewsky (7), who used the stromuhr on the legs of dogs. The accuracy of the method, provided the technical difficulties have been overcome, depends upon the complete occlusion of the venous outflow without obstruction to the arterial inflow. Success in meeting these requirements was repeatedly demonstrated.

The increase in volume flow of blood through the sympathectomized hands of patients, which accompanied elevation of local temperature, was reported in a previous communication (1). The rise in blood flow found in the present experiments on dogs (figs. 1 and 2) is in agreement with the observations made on human cases.

Wide variations in circulation were encountered in the same paw on different days even though the bath temperature was approximately the same. In dog S (expt. 4, table 1), the blood flow at 36.9°C. was 7.5 cc. per minute. On the following day (expt. 3, table 1), although the bath temperature was lower, 33.6°C., the blood flow was greater, 17.5 cc. per minute. We were unable to correlate these variations in volume flow with pulse rate, blood pressure, carbon-dioxide content or pH of the arterial and venous blood.

In spite of the increase in bath temperature and volume of circulation, the differences in oxygen, carbon-dioxide and hydrogen ion concentration of the arterial and venous bloods *in any one experiment* (fig. 1), remained almost constant within the limits of the experimental methods. The results, summarized in table 1, illustrate the narrow range within which these values varied.

The high saturation of the venous blood with oxygen, which was consistently found at all temperatures studied, is at variance with the results reported in the literature. In human subjects, a low venous oxygen saturation has been found when the blood was taken from the arm exposed to temperatures between 18 and 30 degrees C. (8) (9). Our results are probably due to the absence of vasoconstriction of reflex nervous origin. In one dog (H), with intact vasomotor innervation, the oxygen saturation of the venous blood from the paw was studied in experiments similar to those presented in figure 1 and table 1. The venous oxygen saturation at 22.3°C. was 67.4 per cent. At 29.8°C. it was 69.6 per cent, while at 37.0°C. a value of 74.0 per cent was obtained. These values in the blood from the normal paw are in accord with those obtained in normal human subjects (8) (9).

The difference in oxygen content of the arterial and venous blood, which was found to average 1.3 volumes per cent in 58 determinations, is only one-fifth that of the average reported in unanesthetized dogs by Doisy and Beckman (10). The range observed in our experiments, 0.07 to 2.83 volumes per cent, is also less than that, 1.40 to 20.20, observed by Himwich and Castle (11). The differences between our results and those of other investigators are again probably explained by the absence of sympathetic vasomotor reactions in our dogs.

Although in the sympathectomized paw a more constant oxygen difference was observed between the arterial and venous blood, the small absolute difference exaggerated the technical errors of the methods.

An additional source of error which is of even greater importance, was occasioned by the fact that the blood flow could not be determined at exactly the same time that the arterial and venous samples were obtained. Although the measurements of the blood flow and the taking of the venous blood could be accomplished without apparent disturbance to the animal, it was extremely difficult to obtain the arterial sample of blood without stimulation. The performance of arterial puncture has been noted to cause increase in cardiac output (12) and alteration of respiration (13). In the presence of a high oxygen saturation of the arterial blood, increase in respiration would affect the oxygen content only slightly. In these experiments, however, the oxygen saturation of the arterial blood averaged only 93 per cent in 44 determinations. An illustration of the effect of stimulation on the oxygen saturation of the arterial blood is afforded by experiment 9, table 1. The second sample of arterial blood was taken when the dog was lying quietly on the table with the paw in the plethysmograph. The oxygen saturation of 89 per cent was essentially similar to the first sample taken under the same conditions. The paw was then removed from the plethysmograph and the dog was turned over onto the opposite side. The sample which was then taken had an oxygen saturation which was increased to 94.2 per cent, with no appreciable change in the oxygen capacity. The absolute change in oxygen content was only 0.65 volume per cent, but represented 41 per cent of the total arterio-venous oxygen difference.

The carbon-dioxide content of the arterial blood is even more seriously altered by changes in respiration (13). The variations in the carbon-dioxide difference were greater than the range observed in the oxygen difference between the arterial and venous blood. This variability was shown by the frequency with which no difference or even negative values were found for the arterio-venous carbon-dioxide difference. In 3 of 55 determinations the venous carbon-dioxide was found to be below the arterial.

The oxygen saturation of the venous blood, which averaged 86.9 per

cent, was remarkably constant in these experiments in spite of wide fluctuations of blood flow and metabolism. In the presence of normal vasomotor innervation "any condition that accelerates the metabolism or retards the blood flow through a given tissue will increase the proportion of oxygen removed from the blood in its passage from arteries to veins" (14). After sympathectomy and inactivation of the adrenals, however, the blood flow and metabolism in any one experiment may be decreased or increased without significant alteration of the proportion of oxygen removed from the blood.

It is recognized that elevation of temperature increases the metabolism of tissues (15). The observations presented in figure 5, which demonstrate an increased utilization of oxygen at higher temperatures, are in accord with this fact. Since the blood flow is increased by heat without any consistent variation in the proportion of oxygen removed from the blood, it is logical to ascribe the degree of increase in circulation to the elevation of metabolism. It is known, however, that smooth muscle is relaxed by heat (16). As the lumina of the blood vessels would be increased by a relaxation of the smooth muscle in their walls, it is possible that the increase in blood flow observed at higher temperatures might be the result of the direct action of heat on the vessels; and that the increase in circulation might merely be coincidental to the increase in metabolism. The constancy of the arterio-venous oxygen difference at divergent rates of blood flow makes this explanation unlikely, but no data were obtained to refute it conclusively.

SUMMARY. Plethysmographic determinations of the volume flow of blood through the sympathectomized paws of three unanesthetized, trained dogs in which one adrenal was removed and the other denervated, showed that the circulation varied directly with the temperature of the bath in which the paw was immersed (figs. 1 and 2). The oxygen content, carbon-dioxide content, and pH of the arterial and venous blood were constant within the limits of error of the technique employed. Analysis of the data obtained in 34 experiments indicated that there was no correlation between the arterio-venous oxygen or carbon-dioxide difference and the blood flow or bath temperature (table 1 and figs. 3 and 4); and that the oxygen utilization and carbon-dioxide production varied directly with the temperature of the bath (fig. 5).

CONCLUSIONS

The circulation through the paws of unanesthetized, trained dogs, after exclusion of vasomotor factors, varies directly with the temperature of the bath in which the paw is immersed.

The arterio-venous oxygen and carbon-dioxide differences, and the

pH of the arterial and venous blood are constant in any single experiment, over wide ranges of blood flow and metabolism.

These observations are consistent with the hypothesis that the circulation through regions deprived of vasomotor control is determined by the metabolic needs of the tissues.

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OVARIAN WEIGHT RESPONSES TO MENOPAUSE URINE INJECTIONS IN NORMAL, HYPOPHYSECTOMIZED AND HYPOPHYSECTOMIZED THYROXIN-TREATED IMMATURE RATS[1]

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In 1933 Evans et al. reported on the administration of *a*, alkaline extracts of beef anterior pituitary; *b*, pregnancy urine extracts, and *c*, pregnant mare serum extracts to normal and hypophysectomized immature rats, and came to the conclusion that the ovaries of hypophysectomized rats are less sensitive to gonadotropic substances than those of normal controls. In 1934 Evans, Pencharz and Simpson reported, however, that hypophysectomized rat ovaries were equally as sensitive to the "synergist" as the normal rat ovaries.

During the course of experiments to compare the effects of menopause urine extracts in normal and hypophysectomized immature rats, it was found that at certain dose levels the weight of the ovaries was actually greater in hypophysectomized than in normal rats. The first part of the present report deals with this increased sensitivity, and the second with experiments made in an attempt to determine whether this might be due to the inactivation of the thyroid as a result of hypophysectomy.

A single batch of menopause urine extract, kept in the form of a dry powder, was used in all experiments here reported. It was prepared from pooled samples of menopause urine by the tannic acid precipitation method of Levin and Tyndale (1936) and was carefully assayed on immature mice. As described elsewhere (Levin and Tyndale—in press) the uterine weight of the immature mouse is a sensitive indicator of menopause urine gonadotropic activity.[3] The doses used in the present experiments upon rats are expressed in terms of milligrams of the dried powder. As assayed upon the immature mouse uterus, this powder contained one mouse uterine unit

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[3] The mouse uterine unit has been defined as the smallest amount of the active material which, when administered to 20–22 day old mice in three divided doses at 24 hour intervals, produces a doubling of the uterine weight 72 hours after the first injection.

per 0.12 mgm. Hence the doses given, 1.2, 1.8, 2.4 and 3.6 mgm., represent 10, 15, 20, and 30 mouse uterine units, respectively. All of the rats, both normal and hypophysectomized, were injected once daily for 5 days and killed 24 hours after the last injection.[4] Under these conditions of treatment, 2.4 mgm. (20 mouse uterine units) nearly doubled the average ovarian weight in the normal immature rat. With the exception of the first series, in which several doses were used, all rats herein reported received 2.4 mgm. of the extract powder.

I. *Normal and hypophysectomized rats treated with menopause urine extract.* Two series of immature female rats were used. In the first series, injections were begun in the normal rats at 20 days of age, whereas

TABLE 1

Average body, ovarian and uterine weights of normal and hypophysectomized rats identically treated with an extract of menopause urine

DOSE	UNOPERATED RATS			HYPOPHYSECTOMIZED RATS		
	NUMBER OF RATS	Body weight	Ovarian weight	Uterine* weight	NUMBER OF RATS	Body weight	Ovarian weight	Uterine* weight
mgm.		*grams*	*mgm.*	*mgm.*		*grams*	*mgm.*	*mgm.*
1.2	6	39	12.7	20.9	5	58	14.3	69.0
1.8	7	45	15.3	24.2	3	60	32.9	89.4
2.4	13	44	18.1	42.2	10	58	39.8	86.9
3.6	9	43	36.6**	85.8	6	53	70.2	92.3

* Uterus drained of fluid before weighing.

** Of all ovaries reported in this table, in so far as could be ascertained from gross examination at the dissecting binocular, but a single pair contained corpora lutea. This pair was from an unoperated rat, at the highest dose level, and weighed 72.2 mgm. as contrasted with the average of 36.6 mgm.

the operated rats were hypophysectomized[5] at 27 to 35 days of age and injections were not begun until 5 days later.

The average ovarian weights are given in table 1. It will be seen that the ovaries of the hypophysectomized rats are approximately twice as large as those of the normal animals at all doses except the lowest. Even at this minimum dose, the greater uterine response indicates a markedly greater functional ovarian stimulation in the hypophysectomized rats.

These data were supplemented by a second series in which the hypo-

[4] In a series of 25 normal rats, not included in this paper, to which the menopause urine extract was given at doses of from 0.6 mgm. to 3.6 mgm. (5 to 30 mouse uterine units), the more standard procedure of 3 daily injections, with autopsy at 96 hours, was used. In this group, the ovarian weights were approximately 30 per cent to 50 per cent greater than with the 5-day treatment period with the same total dose.

[5] Criteria of completeness of hypophysectomy included daily weighing, thyroid and adrenal weights, and microscopic examination of serial sections of the pituitary capsule. No animal is included in which there is any evidence of incomplete ablation.

physectomized rats were littermate sisters of the unoperated animals, and the same age at the beginning of treatment. In this series, half the rats in each of five litters were hypophysectomized at 18 days of age. Five days later, at 23 days of age, treatment was begun. Each rat was given a total of 2.4 mgm. of the dried powder, divided in 5 daily doses. The average ovarian weight of 12 unoperated rats was 28.8 mgm., of 13 hypophysectomized rats was 52.0 mgm. Thus, in spite of the fact that the ovaries of hypophysectomized rats undergo a considerable atrophy during the five-

TABLE 2

Ovarian weights of littermate rats all injected at 23 days with the same dose (2.4 mgm.) of menopause urine extract

LITTER	UNOPERATED		HYPOPHYSECTOMIZED AT 18 DAYS OF AGE	
	Body weight	Ovarian weight	Body weight	Ovarian weight
	grams	*mgm.*	*grams*	*mgm.*
No. 35	27, 54**	16.1	28, 28	28.0
	26, 48	14.1	25, 28	27.2
	27, 48	16.9	27, 28	26.6
No. 39	29, 56	24.8		
	27, 56	46.8*	28, 31	45.2
	29, 57	23.0	29, 26	68.3
No. 41	32, 60	37.8*	32, 31	61.0
			35, 36	67.0
No. 42	29, 52	34.4*	27, 31	62.7
	30, 53	56.4*	26, 28	71.4
	28, 56	23.6	36, 32	73.3
No. 43	29, 54	27.3	30, 27	42.2
	30, 54	24.1	29, 31	50.5
			30, 34	52.4
Average.....	28, 54	28.8	29, 30	52.0

* Ovaries contained corpora lutea, as observed at the dissecting binocular.

** At 18 days of age, and at autopsy 10 days later.

day period elapsing between operation and the beginning of treatment, they are over 80 per cent heavier at the end of the experiment than those of normal littermate controls receiving the identical treatment (table 2).

That body weight differences do not influence these results is seen by a comparison of the body weights at autopsy in the two series. In the first series (table 1) the average body weight of the hypophysectomized animals was greater than that of the normal animals, while the reverse was the case in the second series (table 2). In both groups, however, the ovaries of the operated rats were larger than those of the unoperated.

It is important to note that, in so far as can be determined by careful examination at the dissecting binocular, this difference of response in the two types of animal is to be observed only in regard to the follicular phase of ovarian development. As the dosage of menopause urine is increased, grossly observable corpora lutea appear in many of the ovaries of the unoperated animals at far lower dose-levels than those required to produce them in the hypophysectomized rat. Simultaneously with the appearance of such grossly observable corpora, there is a marked rise in the average ovarian weight. Thus, with 4.8 mgm. of the extract powder (a dose higher than any listed in table 1), 6 of 7 unoperated animals had ovaries with corpora lutea. The average weight of these ovaries (86.7 mgm.) was actually greater than that of the ovaries from 5 identically treated hypophysectomized rats (69.1 mgm.) in which no corpora could be seen.

The unoperated rats in the second series were 3 days older at the beginning of treatment than those of the first series. It will be noted in table 2 that 4 of these had luteinized ovaries. The average ovarian weight of these 4 (43.8 mgm.) is over twice that of the remaining 8 unoperated animals, in which no corpora could be seen (21.2 mgm.). This latter figure compares well with the average ovarian weight of the 13 unoperated rats on the same dose in the first series (table 1) in which also no corpora could be seen (18.1 mgm.). In view of the weight increase accompanying luteinization in the unoperated animals, and in view of the fact that luteinization does not occur with this dose of the extract in the hypophysectomized rat, it seems justifiable to compare only the non-luteinized ovaries of both groups in table 2. If this is done, the hypophysectomized rat ovaries are well over twice as heavy as those of the unoperated controls, and the weight difference is more closely comparable with that seen in the first series (table 1), in which, also, only non-luteinized ovaries are compared (with the one exception noted).

II. *The effect of thyroxin injections on hypophysectomized rats treated with menopause urine extract.* As has already been mentioned, the ovarian weights of the hypophysectomized animals at the termination of treatment are absolutely greater than those of the unoperated rats, although the weights of their ovaries were less than those of normals at the beginning of treatment. This indicates that some factor must be at work in the normal animal to prevent the full effectiveness of the injected follicle-stimulating material from manifesting itself. That the factor in question might be the thyroid gland is indicated by the work of others who have studied the effect of thyroid on ovarian responses. In 1931 Loeb and Friedman treated guinea pigs with acid AP extracts. In those animals in which increased thyroid function had been produced, they noted a "hypotypical" condition of the ovaries. In 1934 Fluhmann produced experimental hyperthyroidism in rats and found that this decreased the effectiveness of acid AP extracts in stimulating the ovaries, whereas thyroidectomy was

followed by greater than normal ovarian responses to the same extracts. Leonard (1935, 1936) and others have subsequently confirmed these results.

If thyroidectomy increases the ovarian response to injected gonadotropic material, the greater stimulation of the ovaries produced in hypophysectomized rats by menopause urine extracts might be due to the profound thyroid atrophy induced by hypophysectomy, and the consequent loss

TABLE 3

Ovarian and uterine weights of hypophysectomized rats treated with menopause urine alone (C.U.) and with menopause urine plus thyroxin (0.04 to 0.08 mgm. daily)

All animals received the same dosage of menopause urine (2.4 mgm.)

LITTER NUMBER	AT OPERATION		TREATMENT			
	Age	Range of body weight	C. U. only		C. U. plus thyroxin	
			Ovaries	Uteri	Ovaries	Uteri
	days	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
36	30	56 to 65	28.1	96.1	12.0	102.8
			33.8	101.3	7.8	17.5
40*	35	48 to 56	35.4	96.0	21.2	96.4
47	28	44 to 48	44.3	78.8		
			55.1	90.2	16.6	85.7
			38.9	93.6		
49	35	79 to 82			10.0	25.0
					9.6	88.8
50	33	73 to 85	20.1	93.3	8.7	25.1
			21.6	106.6	15.4	97.0
51	27	62 to 65	47.0	102.6	22.1	104.8
Average			36.0	95.4	13.7	71.4

* It has been suggested (Weichert, 1930) that increased B.M.R. may lead to rapid metabolism and excretion of injected hormones, and thus decrease their potency. Two hypophysectomized rats, belonging to litter no. 40, but not cited in the table, were given the same dose of gonadotropic material and were also injected daily with 10 mgm. per kilo of sodium-dinitrophenol. Their ovaries weighed 48.0 mgm. and 66.3 mgm. respectively. Thus their weight response was not decreased by the dinitrophenol injections.

of an inhibiting effect. If this hypothesis is correct, the replacement of thyroid secretion by injections of thyroxin in such hypophysectomized rats should depress the ovarian response to menopause urine, and thus reproduce the condition seen in the normal animal under menopause urine treatment. The success of this procedure, however, depends upon still another factor, namely, whether or not the effect of thyroid upon the ovarian response is mediated through the hypophysis or in some more

direct manner, since, in the former case, it could not exert its action in the hypophysectomized animal. These questions have been tested in the following experiments by the simultaneous injection of thyroxin and of menopause urine extract in hypophysectomized rats.

Five litters of immature female rats were hypophysectomized at 27 to 35 days of age. Five days after hypophysectomy, each of the animals was injected once daily for 5 days with a total of 2.4 mgm. of the menopause urine extract. One half the rats in each litter were also simultaneously injected in a different subcutaneous area with 0.04 to 0.08 mgm. thyroxin daily, beginning, however, one day before the gonadotropic treatment was started. The animals were killed 24 hours after the last injection. Aside from a slight tremor, no deleterious effects could be observed in the thyroxin-treated rats. Daily weighing gave results no different from those obtained in other hypophysectomized rats. There was no increase in the adrenal weights of the thyroxin-injected animals.

The average ovarian weight of the 9 rats which received only the gonadotropic material was 36.0 mgm., whereas that of the 9 rats receiving also thyroxin was only 13.7 mgm. (see table 3). Thus, in the absence of the pituitary gland, thyroxin injections markedly decreased the follicle-stimulating potency of menopause urine extracts, indicating that this action of thyroxin is not mediated through the hypophysis.

If the dose of the gonadotropic extract is sufficiently increased without a proportionate increase in the amount of thyroxin injected the latter is no longer able to exert the same degree of inhibitory effect. Two litters of hypophysectomized rats (27 days at operation) were given 4.8 mgm. of the menopause urine extract, and half the rats in each litter were also treated with 0.05 mgm. daily of thyroxin. The average ovarian weight of the 5 thyroxin injected animals was 63.9 mgm., while that of the 5 rats not treated with thyroxin was 80.2 mgm.

Discussion. Riess and Perény (1928) reported that the amount of estrogenic substance necessary to induce estrus in castrate rats was increased 3 to 5 times when a pretreatment for 6 days with thyroxin was given. In 1933 Van Horn confirmed this result. Both Weichert (1930) and Van Horn (1931) suggested that the thyroxin might cause an increased metabolism and excretion of estrogenic substance and thus explain the antagonism of Riess and Perény. In our experiments an analogous effect is produced by thyroxin, but a different end-organ, the ovary, is concerned. In both cases the question which presents itself is whether the thyroxin acts on the end-organ in question, or whether, as suggested by Weichert, it alters either the rate of elimination or the nature of the injected hormone. Our data, however, yield no information on this point, indicating only that the hypophysis is not involved. (See footnote, bottom table 3.)

The fact that in hypophysectomized rats thyroxin treatment decreases

the ovarian weight response to injected menopause urine is, of course, only indirect evidence that the thyroid secretion, in normal rats, is responsible for the lesser ovarian stimulation which such animals show in comparison with their hypophysectomized littermate sisters. It is, however, difficult to avoid the conclusion that, since injected thyroxin is capable of producing its effect in the absence of the hypophysis, the physiologically secreted thyroid hormone is probably also acting similarly in the non-hypophysectomized animal. Although such an action would be sufficient to account fully for the differential response of the two types of animals, it is entirely possible that some other mechanism is primarily involved.

The greater ovarian response in the hypophysectomized rat, as contrasted with the normal, menopause urine injected animal, is perhaps analogous to the greater metabolic response found in the hypophysectomized rat by Smith, Greenwood and Foster (1927) upon administration of thyroid. These authors reported that if the same minimal, "physiologic" intraperitoneal doses of fresh sheep thyroid extract were given, the basal metabolic rate was almost four times as great after hypophysectomy as after thyroidectomy, and eight times as great after hypophysectomy as in the normal rat.

SUMMARY AND CONCLUSIONS

1. Doses of menopause urine extract which gave marked follicular enlargement and little or no grossly observable luteinization in the immature rat ovary, resulted in the production of much heavier ovaries in hypophysectomized animals than in unoperated controls.

2. With higher dosage which gave luteinization in the normal but not in the hypophysectomized rats it was found that the ovarian weight response was reversed, the normal ovaries being the heavier.

3. The greater ovarian weight obtained in the hypophysectomized rat during the follicular phase of ovarian development indicates that an inhibitory factor is operative in the normal animal. That this factor could be the greater activity of the thyroid gland is evidenced by experiments in which the ovarian follicle stimulation induced by extracts of menopause urine in immature hypophysectomized rats has been markedly decreased by the simultaneous injection of thyroxin.

4. These latter experiments indicate that the inhibiting action of thyroid hormone upon the mechanism of gonad-stimulation is a direct one and not mediated through the pituitary gland, since the experiments were performed on hypophysectomized animals.

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CONDITIONS AFFECTING THE ABSORPTION SPECTRA OF VITAL DYES IN PLASMA

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Various reports scattered through the literature lead one to believe that the group of dyes generally used for determinations of plasma volume are considerably affected in color by salts and proteins. Since these color changes might conceivably place serious limitations upon the dye-dilution method, the separate and combined effects of salts and proteins on the spectral absorption of several vital dyes have been systematically investigated. Some effort has also been made to use spectrophotometric analysis in identifying certain of these dyestuffs that are sold under non-specific commercial names.

I. Eighteen different dye samples representing at least nine distinct tetrazo dyes have been selected for consideration. Their spectral absorption in 0.001 or 0.002 per cent solution was studied with special reference to the effect of salt (sodium chloride) and protein (plasma or serum). The observations were made with a König-Martens spectrophotometer and with Hardy's recording spectrophotometer[1] (Hardy, 1935). Each dye solution was read against an identical dye-free solvent; the densities given therefore actually represent the absorption due to the dye itself.[2]

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[1] Although Hardy's instrument measures diffuse transmission and the König-Martens spectrophotometer gives only direct spectral density, the results from the two instruments were indistinguishable with clear solutions. The Tyndall effect from the dye was apparently too small to make any appreciable difference in the dilute dye solutions under observation. If, on the other hand, one determines the optical density of a turbid fluid, such as serum displaying marked lipemia, the results are not at all the same with these two types of instruments. The degree of scattering can be measured only in the König-Martens, since in this instrument the undeflected parallel beam is alone transmitted to the photometer. In Hardy's machine nearly all the scattered light is also picked up. This, however, does not interfere with the correct determination of the dye concentration in a turbid solution provided the readings are made against a dye-free solution of exactly the same turbidity, in which case the scattering is the same in both dye-containing and dye-free solution.

[2] The Schultz absorption cell supplied with the König-Martens spectrophotometer was not used for two reasons. First, it does not permit direct cancellation of the

From an accurately prepared 0.25 per cent water solution of each dye, dilutions were made into *a*, distilled water; *b*, 0.9 per cent sodium chloride, and *c*, 16 per cent oxalated plasma or 12 per cent serum made up with 0.9 per cent sodium chloride. The final solutions were adjusted to either 0.001 or 0.002 per cent, depending upon the color strength of the dyestuff, in order to have a convenient optical density in the region of maximal absorption by the dye. The spectral absorption curves from each dye in the three solvents have been placed on the same graph to facilitate direct comparison.

So far as possible, the following information has been collected for each dye studied: *a*, Colour Index number (Rowe, 1924); *b*, the name of the manufacturer and the number of the dye batch; *c*, the supposed structural formula. The structural formulae of some of the common dyes have been omitted from the text, but the reader can find them in the Colour Index.

RED DYES. *Brilliant vital red* (Evans) N. A. C.[3] Colour Index no. 456. The spectral absorption curves obtained with two samples of brilliant vital red are included in figure 1 (a and b) to illustrate the variation in purity frequently observed between different batches of the same dye, although made by the same manufacturer. The maximal density of batch no. 5864 is less than that of no. 2576 by approximately the same amount in all three solvents, but the absorption maxima fall at the same wavelengths for both dyes. This presumably means that no. 5864, the weaker of the two, simply contains more impurity in the form of some inert non-coloring matter.

Sodium chloride (0.9 per cent) causes marked reduction (20 to 30 per cent) in the color intensity of brilliant vital red and also shifts the peak of spectral absorption slightly toward the shorter wavelengths (from 502 to 498 millimicrons) (see fig. 1). The percentage decrease in density upon addition of salt varies with the batch which is used and probably depends upon the amount of salt present in the original dyestuff. In plasma diluted with saline, the color intensity is greater than in saline alone but still very much less than in water. This is contrary to the report of

absorption due to the solvent which must therefore be read separately and subtracted from the absorption of dye plus solvent. Second, if the dye is present in water or saline, it precipitates on the frosted surfaces and in the cracks of the Schultz cell with the result that successive readings do not check. Addition of plasma prevents the precipitation of the dye. Paired quartz cells with optically ground endplates eliminate both of these difficulties. They may be obtained in 5, 10 and 20 mm. depths from Macalaster, Bicknell and Co., Cambridge, Mass. In these cells there is no precipitation of dye from saline solutions. Quartz is preferable to glass since it is not readily scratched. Furthermore, since fluid drains from quartz without leaving droplets adhering to the surface, it is often unnecessary to rinse the cells between samples provided these are nearly the same concentration.

[3] National Aniline & Chemical Company.

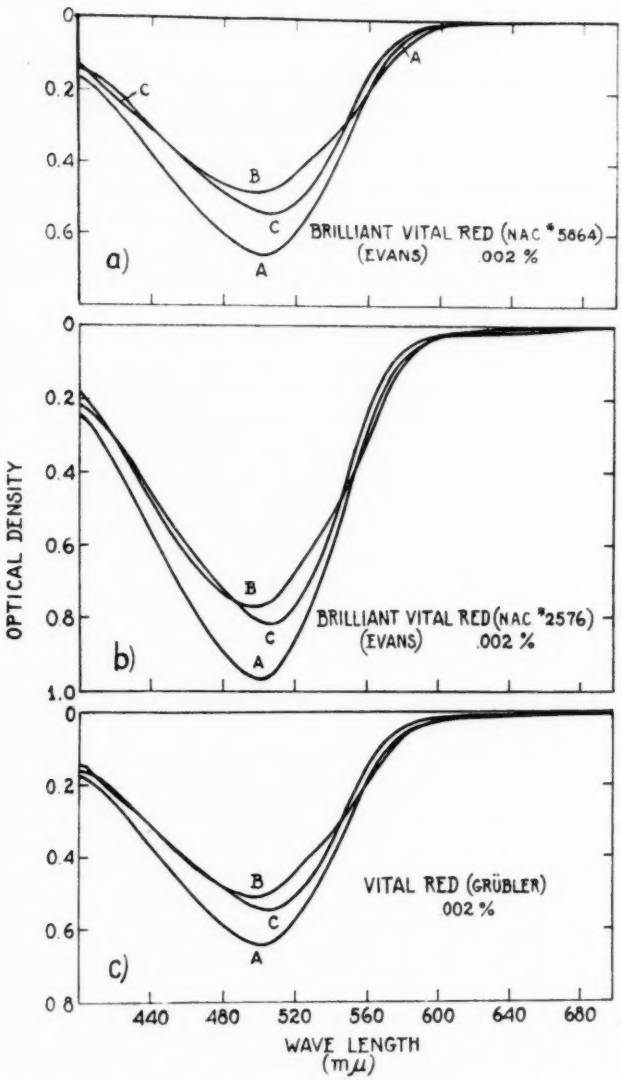


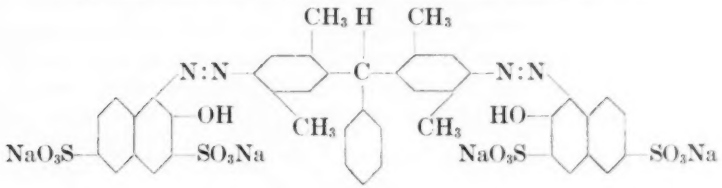
Fig. 1. In figures 1 to 9 inclusive the spectral absorption curves are labeled *A*, *B*, *C*, according to the solvent used. *A*, distilled water; *B*, 0.9 per cent sodium chloride; *C*, oxalated plasma diluted with 6 parts of 0.9 per cent sodium chloride, or serum diluted with 8 parts of 0.9 per cent sodium chloride.

The curves of brilliant vital red in charts (a) and (b) illustrate the difference in spectral absorption frequently observed in different commercial batches of the same dye. The curves in (c) are from a sample of vital red procured from Grübler & Company in 1934. Their similarities to those in (a) indicate that in spite of the difference in name these two dyestuffs are identical (see text).

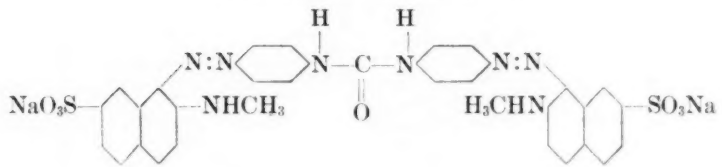
Graff and Clarke (1931) who found that the density of brilliant vital red is higher in plasma than in water. The peak of the curve shows a small but definite shift toward the longer wavelengths (from 498 to 507). Therefore, even in a colorimeter which cancels the plasma color (Bürker), the saline and plasma solutions will not match.

Vital red.

Monosol carmine L. S., B. D. C.[4] Colour Index no. 357



"*Vital neurot*," Grübler & Co.



The variety of structural formulae which have been given for vital red at once indicates that this name does not apply to a specific dyestuff but has been used indiscriminately for several different substances. The formula given by Keith, Rowntree and Geraghty (1915) corresponds to Colour Index no. 357. Dawson, Evans and Whipple (1920) apply the name vital red to two substances of different structure; one of these, Rowntree II no. 273, corresponds to Colour Index no. 436 and is known also as acetopurpurin 8B. The formula for Grübler's vital red published by Lindhard (1926) is, according to information from the manufacturer, the formula for what is now known as "vital neurot". However, this formula differs somewhat from the one which Evans (1921) gives for vital new red. Fleischer-Hansen (1929) working in Lindhard's laboratory employed a vital red, which according to the formula supplied in one of his papers (1929) is the same as the original vital red used by Keith, Rowntree and Geraghty (1915). Spectrophotometric analysis of a sample of Fleischer-Hansen's dye, obtained through the courtesy of Dr. H. Christensen, suggests that it is essentially the same dyestuff as monosol carmine L S which, moreover, is also given the same formula and is listed as no. 357

[4] British Dyestuffs Corporation.

in the Colour Index (cf. a and b, fig. 3). The blood volume results of Fleischer-Hansen (1930) are so much at variance with those of other workers and even with those of Keith et al. (1915), who presumably used the same dye, that it seemed desirable to ascertain from the absorption curves whether or not this difference could be due to his use of water standards (see below).

A sample of vital red obtained from Grübler in 1934 yields spectral absorption curves (fig. 1c) which are so similar to those obtained with brilliant vital red (Evans) (batch no. 5864, fig. 1a) that one suspects the dyes are identical. In the plasma solution, the curves of these two dyes are indistinguishable, both with respect to the point of maximal absorption (507 millimicrons) and the density throughout the visible spectrum. Indeed, Grübler & Co. (private communication) give their vital red a

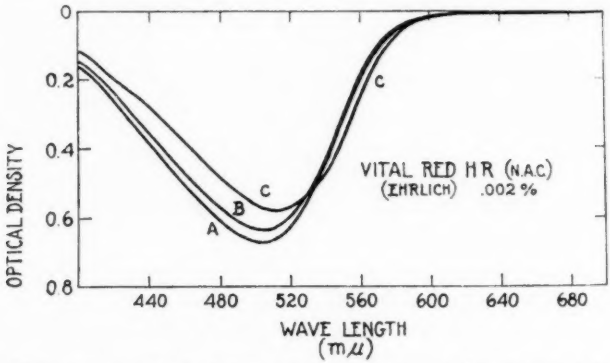


Fig. 2. From a comparison of these curves with those in figure 1c and 3a it is apparent that the name vital red is not specifically applied to one dye.

formula corresponding to Colour Index no. 456 and hence identical with that of brilliant vital red (Evans).

But vital red H R (Ehrlich) from the National Aniline and Chemical Company (fig. 2) is apparently not the same dye as brilliant vital red. The difference is recognized especially in the spectral absorption curve of the saline solution which at the peak is only slightly lower than that of the water solution. Also, it may be seen that the peak of absorption remains at the same wavelength in both water and saline (504 millimicrons). Furthermore, the peak is higher in saline than in plasma, whereas the reverse holds for brilliant vital red (N.A.C.) or Grübler's vital red (fig. 1).

Evidence that the vital red used by Fleischer-Hansen (1929) corresponds to Colour Index dye no. 357 (monosol carmine L S) may be found in figures 3 (a, b) and 4. Comparison of the absorption curves of these two dyes

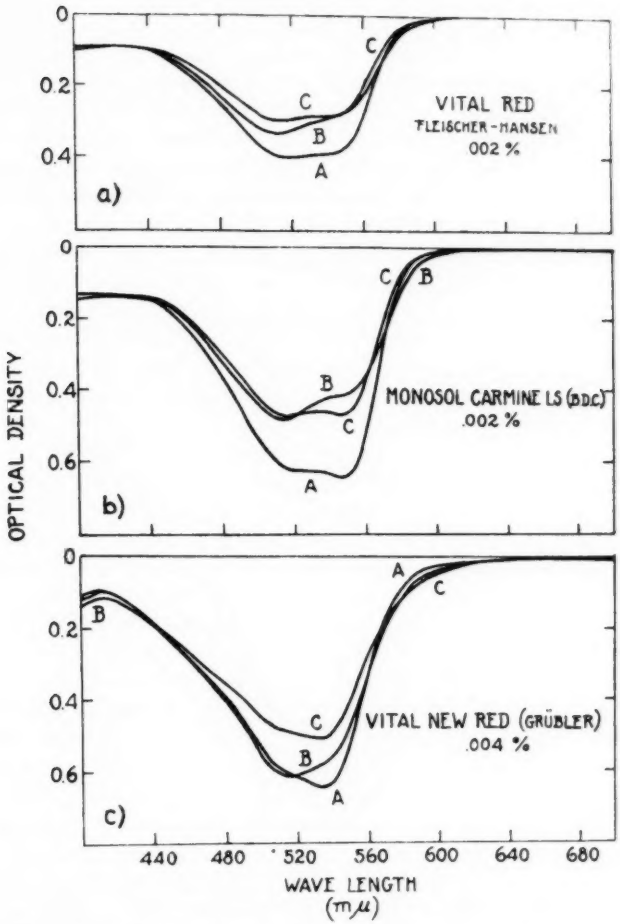


Fig. 3. The curves in (a) were obtained from a sample of the batch of dye used by Fleischer-Hansen (1929) for plasma volume determinations. The similarity in the shapes of curves *A* and *C* shows why this investigator was able to match dye-tinged plasma against water standards, but the difference in height of the two curves indicates that the values for plasma-dye concentration obtained by using a water standard are incorrect.

(b) Monosol carmine LS, Colour Index no. 357. According to its formula in the Colour Index this dye is identical with the vital red originally used by Keith, Rowntree and Geraghty (1915).

(c) Vital new red. The formula given for this dye by the manufacturer (Grübler) indicates that it is the same dye which Lindhard (1926) used.

in the same solvents reveals similar contours (fig. 4) and absorption maxima at the same wavelengths. The ratio of the color strength in

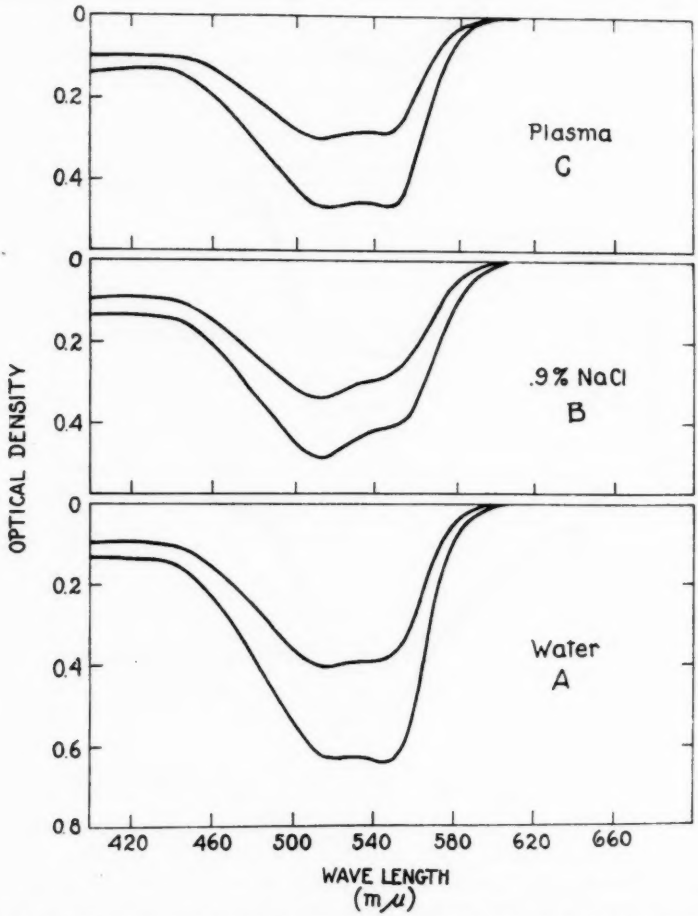


Fig. 4. Direct comparison of the spectral absorption curves of the vital red used by Fleischer-Hansen (1929) with those of monosol carmine LS (Colour Index no. 357), showing that the only essential difference between these dyestuffs is in their color strength. In each case the upper curve is Fleischer-Hansen's sample and the lower, monosol carmine LS.

plasma to that in water solution is the same (75 per cent) for both dyes (cf. figs. 3a and b). The color strength of Fleischer-Hansen's vital red is 62 to 64 per cent as great as that of monosol carmine L S in both water

and plasma solutions (cf. figs. 4a and c). Finally, 0.9 per cent sodium chloride alters the absorption curve of both dyes in the same characteristic manner (fig. 4b). It is therefore highly probable that these two dyestuffs are essentially the same. Lack of equality in color strength may be due simply to color-free impurities.

From the spectral absorption curves in figure 3a, it should be possible to estimate the error which Fleischer-Hansen introduced in his plasma-volume determinations by using water standards. Since the absorption curves of the dye in water and plasma solutions have the same shape, these solutions can be matched satisfactorily in a compensating colorimeter; but inasmuch as the plasma curve is 25 per cent lower than the water curve throughout the region of maximal density, an attempt to estimate plasma-dye concentration by comparison with a water standard will yield a result only 75 per cent of the true value. The calculated plasma volume will therefore be too high. Unfortunately, Fleischer-Hansen (1930) did not report the plasma volumes but only the total blood volumes; hence no corrections can be made on the basis of his published results. As a matter of fact, if the correction for using water standards were applied it would reduce his calculated plasma volumes and only serve to accentuate the difference between his results and those of Keith et al. (1915) and others.

It was pointed out above that vital new red (Grübler) corresponds to the vital red that Lindhard (1926) used. The similarity in the contours of the absorption curves from plasma and water solutions (see fig. 3c) accounts for Lindhard's success in matching dye-tinged plasma with water standards, but the difference in optical density of the dye in these solutions also opens his results to serious question. This applies not only to his plasma-volume determinations but also to his extensive experiments on the absorption of dye by the erythrocytes. The error arising from the use of water standards appears to be in the same direction and of the same order of magnitude (20 to 30 per cent) as with the vital red employed by Fleischer-Hansen (1929).

Congo red, N. A. C. Colour Index no. 370. Congo red was for some time the center of a controversy over the use of water standards in plasma-volume determinations (Griesbach, 1921, 1928; Schmidt, 1927; Seyderhelm and Lampe, 1925; Heilmeyer, 1929; Smith, 1930). The spectral absorption curves of this dye in water, saline and plasma (fig. 5) at once explain why the water and plasma solutions cannot be matched in a colorimeter (Lindhard, 1926; Fleischer-Hansen, 1929) and why the plasma-dye concentration determined by comparison with water standards is incorrect. In water solution, congo red has a maximal absorption at 499 millimicrons; in 0.9 per cent sodium chloride the peak is shifted to 487 millimicrons and the density is 17 per cent lower than in water; in plasma the peak

falls at 508 millimicrons with the density 13 per cent lower than in water. Comparison of these results with Heilmeyer's (1929) spectral absorption curves for "wasseriger" and "serumkongorotlösung" leads one to the conclusion that his "water solution" was really saline. In this so-called "water solution" he found the absorption maximum at 490 millimicrons, in serum it appeared at 510 millimicrons; the optical density in "water" was 4 per cent lower than in serum. Reference to figure 5 shows that his results on the so-called "water solution" agree with ours on saline, both in respect to wavelength at which the peak of absorption occurs and to the density relative to density in serum solution. That Heilmeyer's "water solution" contained salt is furthermore shown by his report that

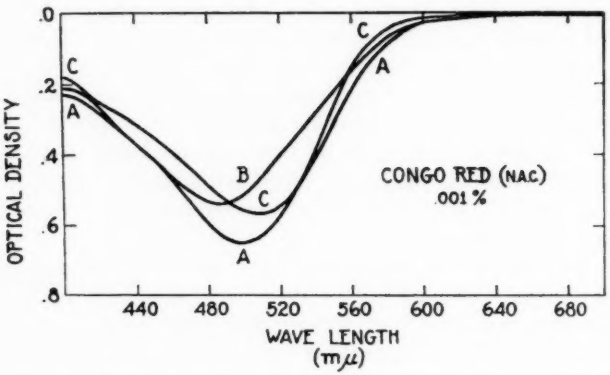


Fig. 5. Congo red, Colour Index no. 370. According to the spectral absorption curves shown here the use of water standards (Griesbach, 1921; Schmidt, 1927) introduces a considerable error in plasma volume determinations made with congo red.

the addition of $NaHCO_3$ for the purpose of adjusting the pH to that of serum did not alter the absorption curve.

This confusion with regard to what is meant by a water standard obviously makes considerable difference in interpreting the results of colorimetry involving comparison of plasma samples with such water standards. On the basis of spectral absorption maxima (fig. 5) it may be seen that use of a water standard should yield a plasma-dye value which is far too low, whereas with a saline standard the result should be slightly too high. But with solutions that cannot be perfectly matched, the judgment of the observer and the particular light filter used may also affect the outcome of the colorimetry in a manner which is unpredictable.

Blue dyes. As a group, the dyes trypan blue, niagara sky blue,

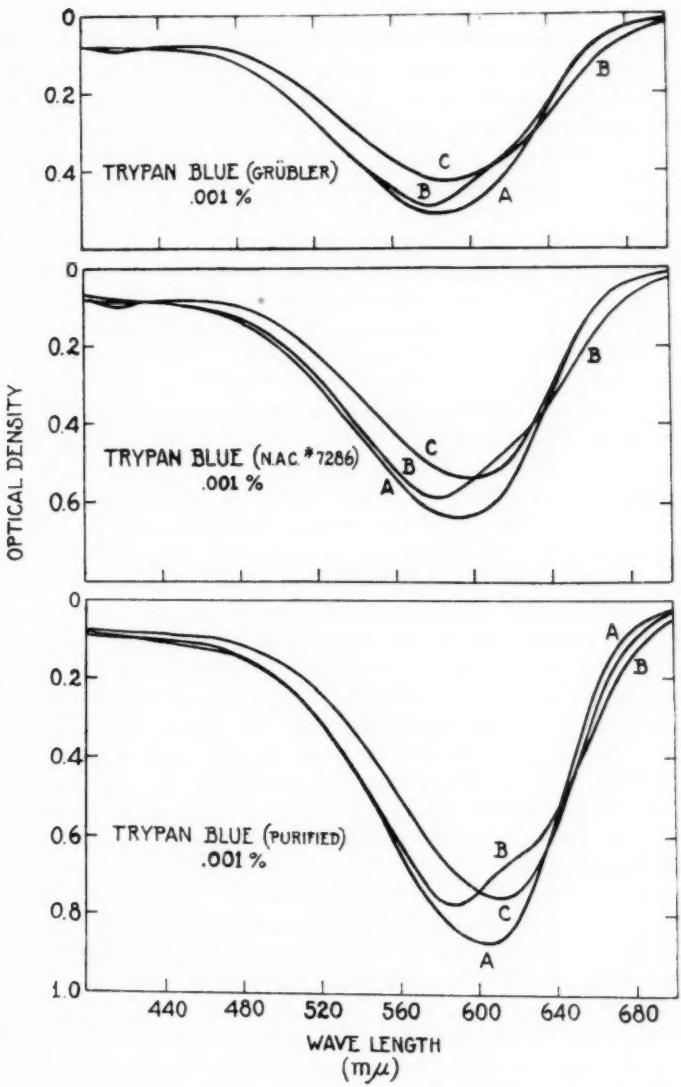


Fig. 6. Trypan blue, Colour Index no. 477. Showing the spectral absorption curves obtained from two commercial samples and from one which has been purified by Doctor Hartwell.

niagara sky blue 6B and T-1824 are interesting because there seems to be a definite relation between their structure and the rate at which they are lost from the blood stream (Gregersen, Gibson and Stead, to be published). Since their spectral absorption maxima in plasma fall between 600 and 650 millimicrons, well beyond the hemoglobin bands, these dyes are colorimetrically ideal for plasma-volume determinations (Gregersen, 1937), but the first three leave the plasma so rapidly that a significant amount of the injected dye disappears before complete mixing occurs.

Trypan blue, N. A. C. Colour Index no. 477. Three samples of trypan blue were subjected to spectrophotometric analysis (fig. 6). Two of these were commercial products; one came from Grübler & Co. and the other from the National Aniline and Chemical Company. The third was a sample purified by Doctor Hartwell. The capillary test showed that the commercial products contained a red component. When this was removed, together with inert salts, the dyestuff not only had a greater color strength but the absorption maxima in water and plasma occurred further in the red. It may be seen that the absorption curve in saline failed to show an equal shift with purification of the dyestuff.

Niagara sky blue, N. A. C., Batch no. 2102. Colour Index no. 520. This dye is a combination of one mole of dianisidine with two moles of 1-amino-8-naphthol-3:6-disulphonic acid (H-acid). Structurally it differs from trypan blue only in having OCH_3 instead of CH_3 groups attached to the diphenyl nucleus. The spectral absorption curves of this dye in water, 0.9 per cent sodium chloride and plasma (fig. 7b), as well as the relation of these curves to one another, are strongly reminiscent of trypan blue. Indeed, except for the difference in ordinates, the curves of trypan blue (fig. 7a) could be superimposed upon those of niagara sky blue; the absorption maxima occur at the same wavelengths, and salt and plasma introduce the same modifications in the absorption curves of both dyes. From these observations it appears that the substitution of the OCH_3 for the CH_3 groups in the nucleus makes very little if any difference in the spectral absorption.

Niagara sky blue 6B, N. A. C. (May, 1934). Colour Index no. 518. Niagara sky blue 6B is also a dianisidine dye; but in this case the dianisidine is combined with two moles of 1-amino-8-naphthol-2:4-disulphonic acid (chicago acid). It therefore differs from niagara sky blue only in having the sulphonic acid groups shifted from the 3:6 to the 2:4 positions. Figure 7c shows that this structural change is attended by marked alterations in the spectral absorption properties. The maxima have moved approximately 30 millimicrons further into the red, which accounts for the more intense blue color of this dye in comparison with niagara sky

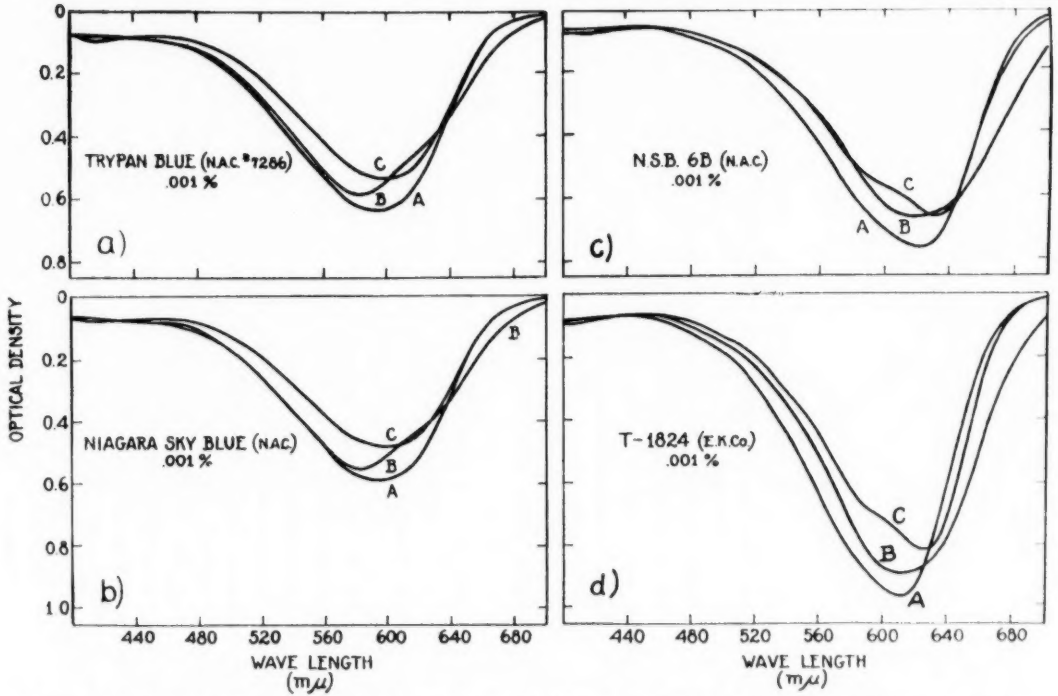
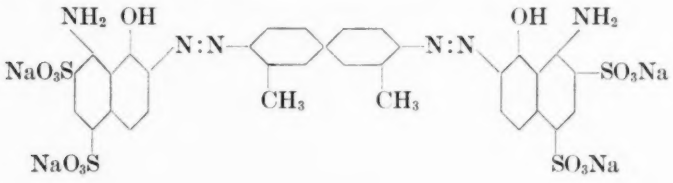


Fig. 7. (a) Trypan blue, Colour Index no. 477. (b) Niagara sky blue, Colour Index no. 520. (c) Niagara sky blue 6B, Colour Index no. 518. (d) T-1824. Comparison of the curves in (a) and (b) in relation to the structural formulae indicates that the change from the tollidine to the dianisidine form does not affect the spectral absorption. On the other hand, if the sulphonic acid groups on the naphthalene rings are changed from the 3:6 to the 2:4 positions (compare formulae of trypan blue and niagara sky blue with those of niagara sky blue 6B and T-1824) the spectral absorption peaks are shifted toward the red and the absorption curves in plasma now show an inflection at 600 to 620 millimicrons. The shapes of the curves in saline have also changed somewhat.

blue, and the absorption curve in plasma solution now displays an inflection at 600 to 620 millimicrons.

T-1824. E. K. Co. (1935).



It was observed above in comparing trypan blue with niagara sky blue that the change from the tollidine to the dianisidine form had no distinct effect upon the spectral properties. This is again illustrated by comparison of niagara sky blue 6B with T-1824, a dye which is made by combining tollidine with two moles of chicago acid. Figure 7d shows that this change has only caused a small shift in the absorption maxima back toward the shorter wavelengths and altered the height of the sodium chloride curve with relation to the plasma and water curves. In respect to general contours, the curves are the same, and in particular it should be noticed that the plasma curve shows the same inflection at 600 to 620 millimicrons as did the plasma curve of niagara sky blue 6B. This inflection therefore seems to be a function of the 2:4 positions of the sulphonic acid radicals on the naphthalene rings.

T-1824 could not be found in the Colour Index, although the dye was made some years ago by at least two manufacturers.[5, 6] When the supply made available by Dr. H. M. Evans was used up, Doctors Hartwell and Fieser (1936) of Harvard University synthesized a new batch from which all traces of salt and contaminating isomers were removed. Subsequently other samples have been procured from Germany and also from Dr. Wm. Hartman of the Eastman Kodak Company, who prepared the dye according to directions supplied by Doctor Hartwell. Spectrophotometric analysis showed that the dye was identical with the Hartwell-Fieser preparation.[7] The absorption curves of these various preparations of T-1824 in plasma solutions are collected in figure 8. Nos. 1 and 2 (fig. 8) apparently contain large amounts of inert salt but no other coloring matter, whereas no. 19 seems to be contaminated with a red component.

[5] Meister, Lucius and Brünning.

[6] Industrielle Gesellschaft.

[7] Another batch of T-1824 obtained recently from the Eastman Kodak Company is not as pure as the original preparation. Its spectral absorption in plasma is approximately the same as that of dye no. 3 in figure 8.

II. In view of the fact that salt and protein have such marked effects upon the spectral absorption of the dyes considered above, one might suppose that these dyes would be greatly affected by changes in the

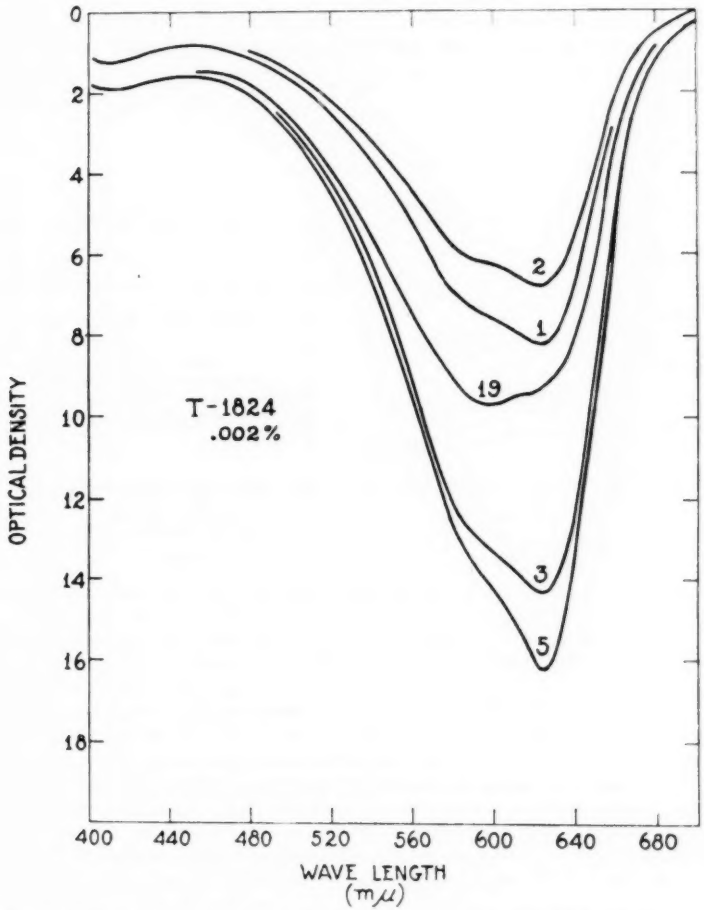


Fig. 8. Spectral absorption curves of different batches of T-1824 (0.002 per cent in plasma solution). *1*, Meister, Lucius and Brünning (old dye). *2*, Sandoz (old dye). *3*, Meister, Lucius and Brünning (new dye, 1934). *19*, Industrielle Gesellschaft (1934). *5*, Hartwell-Fieser (1934).

The batch of T-1824 prepared in 1935 for one of us (M.I.G.) by Doctor Hartman of the Eastman Kodak Company, is identical with the Hartwell-Fieser dye, but a recent batch obtained from the Eastman Kodak Company is not as pure. Its absorption curve is approximately the same as that of no. 3 in the above figure.

composition of the circulating plasma and therefore entirely unsuited for plasma-volume determinations. This, however, is not invariably the case since, as we shall see, the plasma itself may stabilize certain dyes against changes in their spectral absorption.

A few observations on the effect of varying the salt concentration in aqueous solutions of T-1824, vital red, congo red, brilliant vital red are presented in figure 9. In each case the optical density was determined

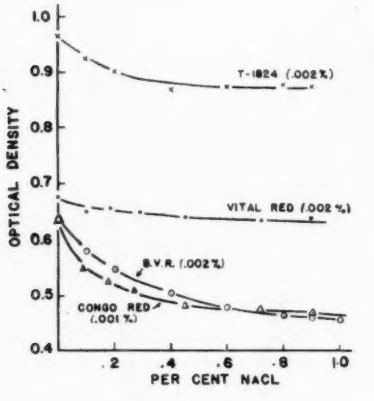


Fig. 9

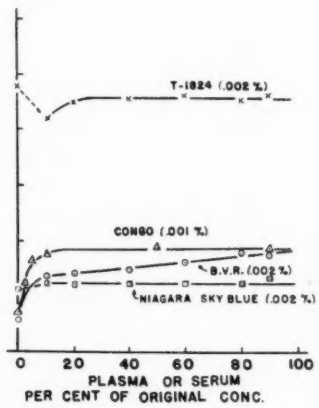


Fig. 10

Fig. 9. Showing the effect of varying the sodium chloride concentration of aqueous solutions of T-1824 (M.L.B.), vital red (N.A.C.), brilliant vital red (N.A.C. no. 5864), congo red (N.A.C.). The optical density of each dye determined at the wavelength where it shows maximal absorption in 0.9 per cent saline (see figs. 1, 2, 5 and 9).

Fig. 10. Showing the effect of reducing the plasma protein concentration on the optical density of T-1824 (M.L.B.), congo red (N.A.C.), niagara sky blue (N.A.C.) and brilliant vital red (N.A.C. batch no. 5864) in oxalated plasma or serum. Determinations made at the point of maximal absorption in plasma (see figs. 1, 5, 7b and 8, No. 1. It may be seen that the plasma can be diluted (with 0.9 per cent NaCl) to about 20 per cent of its original concentration as regards protein without affecting the optical density of T-1824, congo red and niagara sky blue, whereas brilliant vital red is less stable in the range of dilutions shown here.

at the wavelength where the dye in 0.9 per cent sodium chloride displayed maximal absorption. The figures do not therefore necessarily represent the optical density at the peak of spectral absorption in each concentration of sodium chloride. Analysis of the data on brilliant vital red showed that the optical density falls off in logarithmic fashion with the increase in salt concentration up to 1.0 per cent. As more salt is added a point is finally reached (3.5 to 4.0 per cent NaCl) where the dye pre-

cipitates, but if a small amount of plasma is now added, the dye goes back into solution. Further increase in the salt concentration fails to bring down dye until the protein is also precipitated. This behavior is also characteristic of T-1824 except that the NaCl concentration must be raised to about 8 per cent before precipitation occurs from the protein-free solution. The observations suggest that these dyes combine in some

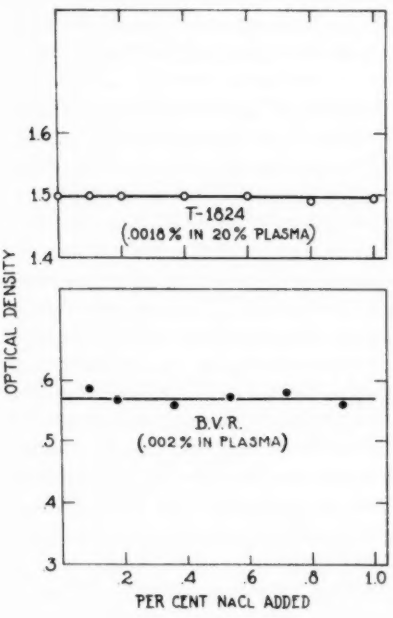


Fig. 11

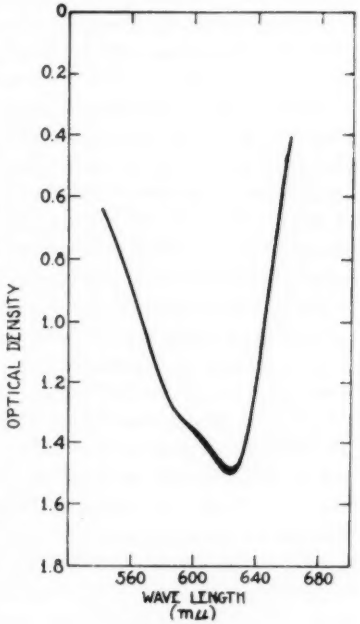


Fig. 12

Fig. 11. Showing that the addition of sodium chloride (up to 1.0 per cent) to plasma solutions of T-1824 and brilliant vital red does not affect the optical density of these dyes. Compare with the effect of salt in the water solutions (fig. 9).

Fig. 12. Further evidence showing that plasma stabilizes T-1824 against salt effect. A series of spectral absorption curves recorded on the same chart with Hardy's self-recording spectrophotometer (1935). Concentration of dye, 0.0018 per cent; plasma, 20 per cent; sodium chloride, ranging from about 0.1 to 3.0 per cent.

way with the plasma proteins. Indeed, it would be difficult on any other basis to account for the peculiarly slow disappearance of T-1824 or brilliant vital red from the blood stream.

Observations on the effect of diluting the plasma proteins, the salt concentration being kept constant, are shown in figure 10. We see here that T-1824, congo red and niagara sky blue undergo no significant change

in optical density until the plasma has been diluted to 20 per cent or less of its original concentration. Consequently we may conclude that even large variations in the concentration of circulating protein can be ignored so far as any effect on the optical density of these dyes is concerned. Brilliant vital red appears to be less satisfactory in this respect. Contrary to the reports by Smith (1930) and Graff and Clarke (1931), the optical density of this dye is not entirely independent of the protein concentration but shows a gradual change throughout the range of plasma dilutions studied. Therefore, standard solutions of this dye should not be prepared in diluted plasma.

It was pointed out above that the solubility of both T-1824 and brilliant vital red in salt solution is greatly increased by the addition of plasma. This well-known protective action of protein has been demonstrated also by spectral absorption measurements, the results of which may be seen in figures 11 and 12. In the latter experiment on T-1824, addition of enough sodium chloride to increase the salt concentration by 3 per cent failed to depress the optical density significantly or even to alter the shape of the spectral absorption curve. It is safe to conclude from these observations that the most extreme variations in the sodium chloride content of the circulating plasma which may be encountered do not affect the spectral absorption of T-1824. It should be pointed out that this does not preclude the possibility of other changes in the plasma interfering with the stability of this or any other dye used to determine plasma volume, and in the clinical application of the dye method pathological bloods must, of course, be tested with respect to their effect on the dye used. Changes in the hydrogen ion concentration of the plasma can be excluded, at least in the cases of brilliant vital red, congo red and T-1824. In the range from pH 7 to 7.7 (phosphate buffers of the same ionic strength) these dyes show no significant change in optical density. As a matter of fact, the spectral absorption curves of T-1824 at pH 5 and 8 are indistinguishable.

No mention has so far been made in this paper as to whether or not the dyes under consideration behave according to the Lambert-Beer Law. Several dyes, in particular T-1824 and brilliant vital red, have been tested repeatedly in this respect and it was found that a strict proportionality exists between the optical density and concentration of the dyes, *provided the composition of the solvent is kept constant.*

III. It has been noted elsewhere (Gregersen, 1937) that the determination of vital dyes in plasma is beset by difficulties arising from the natural plasma color, from residual dye and from hemolysis. The observations reported above show that the effect of the solvent on the spectral absorption of these dyes must also be given due consideration in colorimetry.

In the dye method for determining plasma volume, as originally devised by Keith, Rowntree and Geraghty (1915), care was taken to prepare the standard and unknown in the same dilution of plasma constituents. This was done for the express purpose of cancelling the natural plasma color, but it is now evident that this precaution also served to rule out errors in colorimetry which would otherwise have appeared as a result of differences in the salt and protein effects in the standard and unknown. With compensating colorimeters, it was no longer necessary to prepare the standard in plasma in order to cancel the natural plasma color (Gregersen, 1937). Certain investigators therefore simplified their procedure by using water standards (Griesbach, 1921, 1928; Lindhard, 1926; Fleischer-Hansen, 1929–30, and others) without taking into account that vital dyes do not as a rule display exactly the same color in water as in plasma, a fact pointed out by Seyderhelm and Lampe (1925), Heilmeyer (1929), Smith (1930), and Graff and Clarke (1931). The oversight is a little surprising since salts and proteins have long been known to affect indicator dyes (Sörensen, 1909; Clark, 1928). From the spectrophotometric data presented in this paper, it may be possible to reëvaluate the plasma-volume studies which were done with other than plasma standards.

It is also clear from our studies that spectrophotometric analysis is not only a useful and accurate method of comparing the purity of various batches of the same commercial dyestuff, but frequently enables one to discover if dye samples carrying the same name are actually the same dye. During the past twenty years various investigators have employed so-called "vital red" for plasma-volume determinations without indicating or perhaps even knowing that they were sometimes using dissimilar dyes. A systematic study of all these samples, and other dyes for that matter, which have been used for blood-volume determinations would go far in accounting for the lack of uniformity in the results obtained by different workers.

SUMMARY AND CONCLUSION

1. The purpose of this investigation was to determine precisely what effect changes in salt and protein content of circulating plasma have upon the spectral absorption of some of the vital dyes which have been employed for estimating blood or plasma volume. It is hardly necessary to point out that unless such dyes are stable in the presence of fluctuations in the composition of the blood, the results obtained with the plasma-dye method may be quite misleading.

2. Observations on eighteen dyestuffs, which are believed to represent nine different tetrazo dyes, have been included. At least six of these dyes have been used by various investigators for determining plasma volume. Spectral absorption curves in *a*, water; in *b*, 0.9 per cent sodium

chloride, and in *c*, plasma or serum diluted with 0.9 per cent sodium chloride were obtained with a visual spectrophotometer and with Hardy's (1935) self-recording spectrophotometer (figs. 1 to 9).

3. Addition of sodium chloride to water solutions of these dyes invariably reduces their color intensity (figs. 1 to 9) and, contrary to the reports of Heilmeyer (1929) on congo red and Graff and Clarke (1931) on brilliant vital red, plasma also reduces the color intensity as compared with that of water solution (figs. 1 to 9).

4. In the cases of brilliant vital red, congo red and T-1824, the reduction in optical density of the water solutions of these dyes is proportional to the salt concentration (fig. 9). Variations in the protein concentration also affect the optical density of brilliant vital red slightly, whereas congo red and T-1824 are stable throughout a wide range of plasma dilutions (fig. 10).

5. In plasma solution the spectral absorption of brilliant vital red and of T-1824 is unaffected by large variations in the salt concentration (figs. 11 and 12). Changes in pH between 7 and 7.7 are likewise without measurable effect.

6. From these observations it may be concluded that the most extreme fluctuations in the salt or protein content of circulating blood can be ignored so far as any effect on the spectral absorption of the blue dye T-1824 is concerned. In this respect T-1824 fulfills the requirements for determinations of plasma volume.

A number of the dyestuffs used in this investigation were supplied by the National Aniline and Chemical Company, the Eastman Kodak Company, the Winthrop Chemical Company, Akatos, Incorporated, the British Dyestuffs Corporation, Grübler and Company, Internationalle Gesellschaft and Meister, Lucius and Brünning. The authors also wish to thank Drs. H. P. Smith, H. M. Evans and C. C. Fleischer-Hansen for samples of various dyes, and especially Drs. J. L. Hartwell and L. F. Fieser for preparing T-1824 in a pure form.

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DOES THE NICTITATING MEMBRANE OF THE CAT HAVE A REFRACTORY PERIOD?

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The existence or absence of a refractory period in an effector is of importance in connection with the question of the applicability of the all-or-none law to that effector. Systems which obey that law should be refractory for some time after responding (Adrian, 1914) and, conversely, if a system is not refractory after activation, its responses are graded functions of the stimuli, as opposed to all-or-none reactions.

When two nerve volleys are delivered to the nictitating membrane at an interval smaller than 55 msec., the early component (*I*, cf. Lambert and Rosenblueth, 1935) of the electric response to the second volley is absent (Eccles, 1936). Similarly, if nerve volleys are delivered to the membrane during the period of action of a previous injection of adrenine, the electromyograms are greatly decreased or absent (Rosenblueth and Cannon, 1936; Eccles and Magladery, 1936). The disappearance of the electromyograms in these conditions has been interpreted by Eccles (1936) as denoting a refractory period of the muscle lasting approximately 55 msec.

The electromyograms of the nictitating membrane, however, may not denote all-or-none conducted disturbances (Rosenblueth, Davis and Rempel, 1936) and their absence in certain experimental conditions may, therefore, not necessarily indicate refractoriness. The present study was undertaken to ascertain whether the mechanical responses would confirm or invalidate the inference that the smooth muscle of the membrane has a refractory period longer than that of its nerves. Two series of observations were made. The summation of contraction was recorded when two maximal volleys were delivered at different time intervals. Refractoriness of the muscle for 55 msec. should lead to absent or decreased summation for intervals smaller than the assumed refractory period, and increased summation for greater intervals. This break in the summation curve would be particularly striking after injections of 933F, which, according to Monnier and Bacq (1935) and Eccles (1936), should eliminate the possible masking influence of the chemical mediator.

In the second series of observations nerve volleys were delivered to

the membrane during a response to adrenine. If the hormone should make some of the cells refractory, the response to the nerve volleys should be smaller than that occurring in the absence of adrenine.

METHOD. Cats were used, anesthetized with dial (Ciba, 0.7 to 0.8 cc. intraperitoneally). Isotonic or isometric contractions of the nictitating membrane were studied. The isotonic contractions were recorded by means of a light straw-lever on a kymograph. The isometric contractions were obtained by attaching the membrane to a frictionless torsion-spring optical myograph. The shortening of the muscle in the latter cases was less than 0.5 mm. for the maximal responses of the muscle. The excursions of the beam of light reflected by the mirror in the myograph were either photographed or, more frequently, measured directly on a millimeter ruler placed 1 to 1.5 meters away. The slowness of the responses of smooth muscle makes such direct readings accurate.

As a rule a steady base-line was encountered. When rhythmic movements were present the eye-ball was extirpated and the eye muscles were cut. Exceptionally a small dose of curare was injected. In all the experiments to be reported no eye movements occurred.

Shielded, silver, stimulating electrodes were applied to the postganglionic (experiments reported in section A) or preganglionic (experiments reported in section B) fibers of the cervical sympathetic. The preganglionic strand was cut caudad and usually dissected away from the vagus. In all instances the vagus was cut cephalad and caudad at some distance from the electrodes. When the postganglionic fibers were used the IX, X, XI and XII cranial nerves were dissected and removed for some distance away from the electrodes. The superior cervical ganglion was sometimes crushed; sometimes it was left intact, the preganglionic fibers being cut.

For single- or double-shock activation, break shocks from Harvard induction coils were used, timed by a Lucas pendulum. For repetitive stimulation condenser discharges (capacity 0.05 to 1 μF) were used, timed by hand or by a mechanical interrupter. The stimulating cathode was in these cases proximal to the muscle. The condenser discharges were sometimes sent into the primary of an induction coil, and the diphasic waves from the secondary were used to stimulate. The stimuli were always maximal.

All injections were made into a femoral vein. When different doses of adrenalin were injected into an animal either a constant dilution and variable volume, or the reverse, were employed.

RESULTS. A. *Summation of the mechanical responses to two nerve volleys before and after injections of piperidinomethylbenzodioxane (933F).* The summation of isometric contraction when two nerve volleys are delivered to the membrane at various intervals has been accurately studied by

Brown (1934). He reported that no summation occurred when the interval was less than 2 msec. As the interval was increased the responses grew, first rapidly, then more slowly, to attain a maximum when the interval was about 40 msec. Thereafter occurred a very gradual decline of the tension developed, some summation being still present with intervals as long as 2 sec.

Brown's observations were readily confirmed (cf. fig. 1A). It was further found that when isotonic responses, instead of isometric, were recorded, identical results ensued.

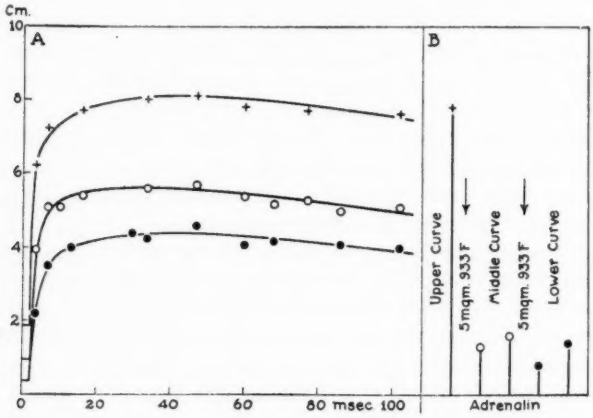

Fig. 1. Isometric responses. Superior cervical ganglion crushed and electrodes on the postganglionic fibers.

A. Ordinates: responses in centimeters. Abscissae: interval (in msec.) between two maximal break induction shocks timed by a Lucas pendulum. Upper curve: before 933F. Middle curve: after 933F (1.2 mgm. per kgm.). Lower curve: after a similar additional dose of 933F.

B. Responses to 0.025 mgm. adrenalin injected as follows: cross, immediately before the first dose of 933F; circles, at the beginning and end of the observations in the middle curve in A; dots, at the beginning and end of the observations in the lower curve in A.

The interest of studying the action of 933F on the summation curve will become clear in the discussion (p. 519). The drug has the property of decreasing greatly the responses of the membrane to adrenine while impairing only slightly those to nerve volleys (Bacq and Fredericq, 1935). The efficiency of the drug was judged by comparing the responses to a standard dose of adrenalin (20 to 50γ) before and after the injections of 933F.

Although the drug depressed even more than was expected the responses to nerve volleys, both twitches and summated effects, the curves of sum-

mation were qualitatively identical with those obtained before 933F. The results were the same for isotonic and isometric responses. Figure 1 illustrates a typical instance. The upper curve was first obtained. An injection of 0.025 mgm. of adrenalin was then made, which elicited a response of 7.8 cm. Five milligrams of 933F were then injected. Two minutes later the same dose of adrenalin elicited a contraction of only 1.3 cm. The middle curve was then constructed. At the end of this series adrenalin was tested. The response was now 1.6 cm., revealing that, although the effects of 933F were not as marked as before, the paralyzing action of the drug was still present. Five more milligrams of 933F were injected. The response to adrenalin decreased to 0.8 cm. The lower curve was constructed. At the end of the experiment adrenalin elicited a contraction of 1.4 cm.

The region of the curves near the interval of 55 msec. was carefully explored and in no instance was any accident or break in the process of summation detected.

B. *Summation of the responses to nerve volleys with those to adrenine.* Rosenblueth and Rioch (1933a) reported that such summation occurs; their study, however, was only qualitative. Eccles and Magladery (1936) have since stated that although nerve volleys elicit further contraction when delivered during the action of the hormone, their effects are greatly reduced, as compared with those which they cause in the resting membrane.

It is not legitimate to judge the efficiency of a stimulus by comparing its action on a resting muscle with that which it exerts during semicontraction—i.e., when the muscle is in a different condition. To obtain a legitimate test the method adopted in the present experiments was to compare the action of nerve stimulation with that of adrenalin both in resting and semi-contracted muscles, as follows.

In some cases (fig. 2) the response (R_1) to a certain frequency of stimulation (F) of the nerve for 30 seconds was matched by that to a dose of adrenalin (A). Doubling the frequency on the nerve (2F) or doubling the dose of adrenalin (2A) yielded approximately equal responses (R_2). It was then found that the response to A + F was R_2. If the muscle had been rendered refractory to the nerve impulses by adrenalin a smaller response than R_2 would be expected.

A more general procedure was the following. Several frequencies of stimulation were applied and plotted against the corresponding responses—a hyperbolic curve ensue (fig. 3; cf. Rosenblueth, 1932b). Several doses of adrenalin were then injected and in turn plotted against the corresponding responses—again a hyperbolic curve ensue (fig. 3; cf. Rosenblueth, 1932a). By selecting proper scales for the abscissae the two curves superimpose satisfactorily (fig. 3). The responses were then

measured to various frequencies applied during the action of various doses of adrenalin. Such responses to the two stimuli, when plotted at the abscissae obtained by adding the dose of adrenalin to the frequency on the common scale invariably fell in the common curve (fig. 3), never significantly below it, as the concept of refractoriness would lead one to expect.

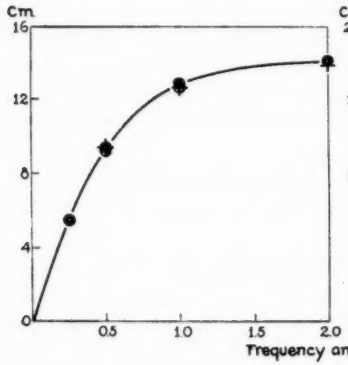

Fig. 2

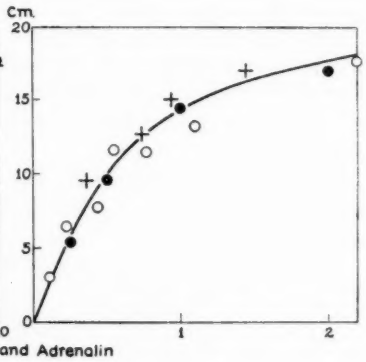

Fig. 3

Fig. 2. Isometric responses. Ordinates: responses in centimeters. Abscissae: frequencies per second of maximal stimulation of the preganglionic fibers and doses of adrenalin injected (unit: 0.22 mgm.). Dots: nerves stimulated for 30 sec. Circles: adrenalin. Crosses: adrenalin injected (dose ½ the abscissa) and, 10 sec. later, nerve stimulated (frequency ½ the abscissa) for 30 sec. The observations in these and the succeeding figures were made in random order. Some of the points are averages of several readings.

Fig. 3. Isometric responses. Ordinates: responses in centimeters. Abscissae: frequencies (per sec.) of maximal stimulation of the postganglionic fibers and doses of adrenalin injected (unit: 0.18 mgm.). Dots: nerves stimulated for 30 sec. Circles: adrenalin. Crosses: adrenalin injected and, 10 sec. later, nerve stimulated for 30 sec. The doses of adrenalin and frequencies of stimulation for the crosses were: 0.018 mgm. and 0.25 per sec.; 0.036 mgm. and 0.5 per sec.; 0.072 mgm. and 0.5 per sec.; 0.072 mgm. and 1.0 per sec.

In these observations, as in those reported in the previous section, the results were qualitatively identical, whether isotonic or isometric contractions were recorded.

Discussion. An appraisal of the value of the evidence presented in relation to the problem of the existence or absence of a refractory period in the nictitating membrane requires some preliminary theoretical considerations.

It is now quite generally recognized that when the sympathetic nerve impulses arrive at the nictitating membrane they cause the liberation of an

adrenine-like chemical mediator and that this mediator can stimulate the smooth-muscle cells to contract. Opinions differ as regards the mode of action of the chemical mediator. The suggestion has been made (see Rosenblueth, 1937) that this action and also that of adrenine is direct, without intervening all-or-none propagated disturbances. On the other hand, Eccles (1936) defends the view that the chemical mediator, and also adrenine, cause contraction by setting up conducted disturbances in the reacting cells.

There is likewise disagreement as to whether the nerve impulses act on smooth muscle exclusively through a chemical mediator, or have an accessory mode of transmission. Monnier and Bacq (1935) and Eccles (1936) claim that in addition to the stimulating effects of the chemical mediator the action potential of the nerve impulses acts as an electrical stimulus to the muscle. Rosenblueth and Cannon (1936) consider the latter mode of activation unnecessary and unlikely.

According to Eccles (1936) the summated mechanical response which occurs when two nerve volleys are delivered at an interval shorter than 55 msec. is explained in the following manner. The first nerve volley stimulates some cells electrically and others chemically. All these muscle cells will be refractory when the second nerve volley arrives, but additional inactive elements will be brought into play by the additional chemical mediator liberated at the nerve endings by this second volley. To test the validity of this explanation it would be necessary to abolish the responsiveness of the muscle cells to the chemical mediator. According to Rosenblueth and Cannon (1936), if this were actually achieved all responses of the muscle would be abolished, since they assume no other stimulus for the muscle. According to Monnier and Bacq (1935) and also to Eccles (1936), however, the drug 933F will have precisely the effect of decreasing or eliminating the action of the chemical mediator while leaving unimpaired the ability of the electrical stimuli delivered by the nerve impulses.

In the experiments reported in section A (fig. 1), summation to two nerve volleys was similar after 933F to that which occurs without the drug. If 933F should have the action attributed to it by Monnier and Bacq and by Eccles, and if the muscle were refractory after the first nerve volley, a second volley arriving less than 55 msec. after the first one should be mechanically ineffective. Not only is this corollary of the assumptions under discussion not fulfilled experimentally, but the ratio of the summated responses to the maximal twitches is greater after 933F than before injections of the drug.

A tissue should not be characterized as refractory unless all its responses are temporarily abolished. The mere absence of *I* potentials does not indicate refractoriness if a mechanical response is present. It may there-

fore be concluded that the observations on which Eccles based his inference of a refractory period were incomplete and that this inference is therefore invalid. It may further be concluded that contraction of the muscle may occur without a preceding *I* potential, a conclusion which had been previously advanced by Rosenblueth, Leese and Lambert (1933).

A similar argument applies to the results presented in section B (figs. 2 and 3). As stated above, according to the dual theory of nerve transmission the responses to nerve impulses which occur after 933F are mainly due to electrical activation of the muscle. Since these responses are only slightly smaller than those of the membrane untreated by the drug, the inference would follow that normally a large part of the responses is due to the electrical mechanism rather than to the chemical one. It would be expected, therefore, that, if adrenine rendered the cells refractory to the electrical stimulus, as Eccles (1936) claims, the responses to nerve volleys during the action of adrenine should be significantly decreased. This expectation, however, is not confirmed experimentally. The nerve volleys are fully as effective with as without adrenine (figs. 2 and 3). It may therefore be concluded that the absence of action potentials when nerve volleys are delivered to a membrane contracted by the hormone is not evidence of refractoriness but evidence that contraction may occur without action potentials.

As opposed to the dual theory, the hypothesis that the transmission of the nerve impulses to the membrane is exclusively by liberation of a chemical mediator readily accounts for the present observations. Two nerve volleys sum their effects—with or without 933F—because the concentration of the chemical mediator is greater than when a single volley is delivered. Stimulation of the nerves when adrenine is present in the muscle increases the concentration of the active substance.

The present results are confirmatory of previous reports which have been questioned by Eccles (1936). The curves correlating the mechanical responses with the doses of adrenalin injected (fig. 3) or with the frequencies of maximal stimulation of the nerves (fig. 3) closely approximate rectangular hyperbolas (cf. Rosenblueth, 1932a, b). Isotonic or isometric recordings of the contractions of the nictitating membrane yield similar results, both qualitatively and quantitatively (cf. Rosenblueth and Rioch, 1933b).

SUMMARY

Isotonic and isometric contractions of the nictitating membrane of the cat were studied in the two following experimental conditions: *a*, application to the postganglionic nerve fibers of two maximal single stimuli separated by variable intervals, before and after injections of 933F; *b*, stimulation of the nerves at various frequencies during the period of action of various doses of adrenalin.

Injections of 933F decrease the contractions of the membrane. The summation curve to two nerve volleys obtained after administration of the drug is similar to that obtained before such administration (fig. 1).

In a partially contracted membrane a given frequency of stimulation of the nerves elicits the same increment of tension whether the initial partial contraction be produced by adrenine or by nerve stimulation (figs. 2 and 3).

The results were similar whether isotonic or isometric recording was employed.

The presence of increased mechanical responses in circumstances in which the electric responses may be absent indicates that the muscle does not have a refractory period of approximately 55 msec., as Eccles (1936) claims. Refractoriness of smooth muscle should not be inferred from electrical evidence alone, without knowledge of the mechanical events. The results are readily explained by the hypothesis that nerve impulses act on smooth muscle exclusively through the chemical mediator.

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THE REVERSIBLE INHIBITION OF MUSCLE GLYCOLYSIS[1]

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Numerous substances have been shown to suppress the glycolytic process and such inhibitory actions have been studied extensively. Only recently has any attention been paid to the possibility of restoration of glycolysis following inhibition. In 1933 Lipmann demonstrated that the inhibition of glycolysis by dichlorophenol-indophenol and oxygen was removed by ascorbic acid and nitrogen. Wagner-Jauregg and Rzeppa (1936a, b) showed that the cupric ion inhibition previously described by Lipmann (1934) may be reversed by glutathione, cysteine, Warburg's coferment or cozymase.

The inhibitory action of heavy metal salts and of oxidizing agents upon enzymes has been shown to be reversible in certain instances. It has been suggested that in a limited number of such cases there may be involved reversible chemical actions upon substituent sulfhydryl groups of the enzyme molecules. The methods of approach and the results of studies in this field have been reviewed by Hellerman (1937). The applicability of such methods to the control of activity of enzymes concerned with the glycolytic mechanism seemed worthy of consideration. The present work involves a study of the suppression of the glycolytic process in extracts of frogs' muscle by means of several organomercuric compounds, of mercuric chloride and of iodine; and of its restoration by certain reagents.

METHODS. Fresh extracts of frogs' (rana pipiens) muscle were used. The muscles of the hind legs of several frogs were removed, weighed and ground in a chilled mortar containing a small amount of sand and water (1 part muscle:1.5 parts water). The resulting mixture was centrifuged and used immediately. Two methods were employed for the determination of the rate of glycolysis: the Warburg manometric method and the chemical estimation of lactic acid. With the Warburg method, solutions prepared as described in the tables were placed in the vessels; 5 per cent carbon dioxide in nitrogen was passed for ten minutes through the vessels while the latter were being shaken in the water bath. The shaking was

[1] A preliminary report of this work was presented at the meeting of the American Physiological Society, Memphis, 1937.

continued for an additional five minutes with the stopcocks open in order to equilibrate the solutions to atmospheric pressure. After the initial readings were made the stopcocks were closed and the glycogen solution was spilled over from side cups. Pressures were observed every five minutes for thirty minutes. When lactic acid determinations (Wendel, 1933) were to be carried out, larger amounts of solutions were taken; the reaction mixtures were shaken for one to two hours in the presence of a gas mixture of the same composition as used previously. For the determination of glycogen the procedure of Good, Kramer and Somogyi (1933) was employed and for the estimation of total carbohydate the method described by Cori and Cori (1933). All reactions were carried out in a water bath at 25° ± 0.02°C.

The mercury compounds used were phenylmercuric hydroxide, p-chloromercuribenzoic acid and mercuric chloride. When phenylmercuric hydroxide (C_6H_5—Hg—OH) was used as an inhibitor, the extract was shaken with a little of this slightly soluble compound for a few minutes and

TABLE 1

Normal glycolysis

DATE: NOVEMBER 19	VESSELS					
	I	II	III	IV	V	Average
Millimeters³ CO_2 in 30 minutes.....	252	245	238	235	234	241

Solution in each vessel: Extract, 1.2 ml.; sodium bicarbonate, 0.4 ml. of a 1.3 per cent solution; glycogen, 0.2 ml. of a 3.94 per cent solution; water, 0.4 ml. Total volume in each vessel, 2.2 ml.

centrifuged in order to remove the undissolved material. The resulting solution was added to the Warburg vessels. p-Chloromercuribenzoic acid (Cl—Hg—C_6H_4COOH) was neutralized with a slight excess of 0.01 N sodium hydroxide before addition to the extract. Mercuric chloride was used in an aqueous solution. Glycogen was used as the substrate in all the experiments. The iodine solutions were made by suitable dilution of 0.1 N iodine solution containing 30 grams of potassium iodide in one liter.

In order to permit sufficient time for reaction of a mercury compound or of iodine with the substances present in the extract before the subsequent addition of a reactivant (e.g., cysteine), the latter was not added until fifteen or twenty minutes after the inhibitor had been mixed with the extract. Under these conditions any recovery was presumably a consequence of reactivation of some component part of the enzyme system and not merely a combination of the inhibitor with the reactivant. That the time of action of the inhibitor was adequate is shown by the resulting inhibition of glycolysis.

RESULTS. After numerous preliminary experiments, control determinations demonstrated that comparable results could be obtained with the manometric method when the same muscle preparation was used in each of five vessels. The amount of carbon dioxide liberated in one-half hour averaged 241 mm.^{3} with a deviation of +11 and −7 (table 1). However, the variations in glycolytic activity of muscle extracts used at different times were so great that the determination of a base line for each experiment was required. Therefore, the results could not be averaged but

TABLE 2

Inhibition of glycolysis with phenylmercuric hydroxide and restoration with cysteine or glutathione

EXPERIMENT	DATE: NOVEMBER 1936	NORMAL GLYCOLYSIS: CUBIC MILLIMETERS CO_2 IN 30 MINUTES			GLYCOLYSIS WITH PHENYLMERCURIC HYDROXIDE: CUBIC MILLIMETERS CO_2 IN 30 MINUTES		
			Glutathione	Cysteine		Glutathione	Cysteine
1	19	266	214		0	372	
2	20	276	236		0	114	
3	23	98		149	0		102
4	27	86		73	0		67
5	28	142	133		14	135	
6	30	208		250	11		69
7	23*	84	72		63	95	
8	24†	158	197		0	0	

Solutions used: Extract, 1.2 ml.; sodium bicarbonate, 0.4 ml. of 1.3 per cent solution; glycogen, 0.2 ml. of 4 per cent solution; glutathione, 0.2 ml. of 5 per cent solution; cysteine HCl, 0.2 ml. of 1.5 per cent solution. Phenylmercuric hydroxide, 15 mgm. shaken with 5 ml. of the extract. Centrifuged, 1.2 ml. used. In the vessels containing cysteine HCl or glutathione there was added extra sodium bicarbonate equivalent to the acidic reagent. Total volume in each vessel, 2.2 ml.

* Trace of the mercurial used.

† Extract with the mercurial was not centrifuged.

only compared as percentage increments from the base line for each individual experiment.

On this basis a series of experiments were made in which part of an extract was shaken with phenylmercuric hydroxide. The glycolytic activity of this extract with and without the subsequent addition of cysteine or reduced glutathione was compared with the glycolytic activity of the normal extract. The results are given in table 2. They show practically complete inhibition of glycolysis in the presence of the mercury compound and partial or complete recovery with cysteine and glutathione. The course of the glycolysis is shown in figure 1 (expt. 5). It is interesting to note that although the final amount of carbon dioxide given off after

reactivation (curve III) is equivalent to the amount obtained from the glycolysis of the normal extract (curve I) the time course is different. Although the data (presented here and elsewhere in this paper) are of limited value for the delineation and comparison of actual rates of glycolysis, they depict, nevertheless, striking differences which apparently reflect well the suppressions and restorations of activity under the conditions stated.

In another series, chemical analyses were made for lactic acid (table 3). The results of these experiments are comparable qualitatively with those obtained manometrically.

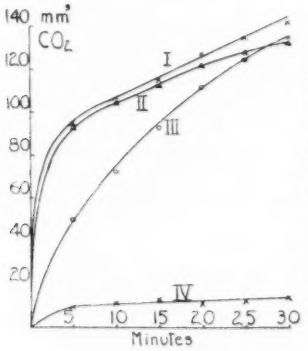

Fig. 1

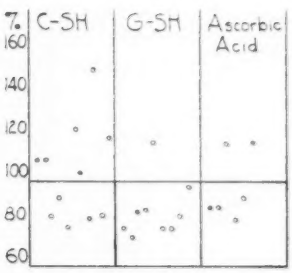

Fig. 2

Fig. 1. Inhibition with phenylmercuric hydroxide and restoration with glutathione. Curve I, glycolysis of normal extract; curve II, glycolysis of normal extract with glutathione; curve III, restoration with glutathione; curve IV, inhibition with phenylmercuric hydroxide.

Fig. 2. Glycolysis with cysteine, glutathione or ascorbic acid added to normal extract. Results given in terms of per cent of glycolysis of normal extract.

The action of glutathione or cysteine on glycolysis of normal extracts was not striking (fig. 2). In a few experiments there was noted an enhancement of glycolysis similar to that found by Geiger (1935) and Gaddie and Stewart (1935).

The results of analogous experiments carried out with p-chloromercuribenzoic acid and mercuric chloride are given in table 4. These substances were also found to inhibit glycolysis; effective restoration was again obtained with cysteine and glutathione. Since p-chloromercuribenzoic acid and mercuric chloride are sufficiently soluble, a qualitative relationship between the concentration and the effects of these substances was observed (table 5; fig. 3). A solution of p-chloromercuribenzoic acid was prepared by addition of 5.6 ml. of 0.01 N NaOH to 20 mgm. of

TABLE 3

Lactic acid determinations

EXPERIMENT	DATE: DECEMBER 1937	TIME OF EXPERIMENT	NORMAL GLYCOLYSIS				GLYCOLYSIS WITH PHENYLMERCURIC HYDROXIDE PRESENT			
			Beginning	Milligram lactic acid. End			Milligram lactic acid. End			
					Glutathione	Cysteine		Glutathione	Cysteine	
		hours								
1	16	1	3.70	4.21		4.50	1.14		1.46	
2	17	1	3.15	4.20	5.05		1.47	1.70		
3	18	2	4.58	6.23	8.57		3.04	3.98		
4	19	2	3.52	4.80		4.25	1.17		2.12	

Solutions used: Extract, 2.4 ml.; sodium bicarbonate, 0.8 ml. of 1.3 per cent solution; glycogen, 0.4 ml. of 4 per cent solution; glutathione, 0.2 ml. of 10 per cent solution; cysteine HCl, 0.2 ml. of 3 per cent solution; phenylmercuric hydroxide, 30 mgm. shaken with 10 ml. of the extract. Centrifuged, 2.4 ml. used; phosphate, 0.2 ml. of a solution of 0.1375 per cent $NaH_2PO_4 \cdot H_2O$ and 0.635 per cent of Na_2HPO_4. Total volume in each vessel, 4.4 ml. In the vessels containing cysteine hydrochloride or glutathione, there was added extra sodium bicarbonate equivalent to the acidic reagent. Definite increments in glycolytic activity were obtained with glutathione and cysteine added to the mercurial inhibited extract.

TABLE 4

Inhibition of glycolysis with p-chloromercuribenzoic acid or mercuric chloride and restoration with cysteine or glutathione

EXPERIMENT	DATE	NORMAL GLYCOLYSIS: CUBIC MILLIMETERS CO_2 IN 30 MINUTES			GLYCOLYSIS WITH MERCURIAL: CUBIC MILLIMETERS CO_2 IN 30 MINUTES		
			Glutathione	Cysteine		Glutathione	Cysteine
1	Dec. 22	115	136		19	149	
2	Jan. 5	151		187	35		210
3	26	259		270	43		251
4	27	181		150	25		113
5	27	249	196		13	206	

Concentration of solution is the same as given in table 2.

Experiment 1. p-Chloromercuribenzoic acid, 20 mgm., was shaken with 5.6 ml. 0.01 N sodium hydroxide. Of the supernatant fluid, 0.2 ml. was used.

Experiment 2. p-Chloromercuribenzoic acid, 20 mgm., was dissolved in 10 ml. of 0.01 N sodium hydroxide and sufficient hydrochloric acid was added to neutralize the excess sodium hydroxide. On neutralization, a precipitate formed. Of supernatant fluid, 0.2 ml. was used.

Experiments 3, 4 and 5. Of 0.1 per cent mercuric chloride solution 0.2 ml. was used in each experiment.

Total volume in each vessel, 2.2 ml.

this substance. After the mixture was shaken for a few minutes the supernatant fluid was decanted and the residue dried and weighed. Therefore, the concentrations of p-chloromercuribenzoic acid given in table 5 indicate approximately the amount of the mercury compound in solution. Suitable controls demonstrated that the slight excess of sodium hydroxide added with the mercury compound introduced no complications. A graphic summary of all of the results with mercury compounds is given in figure 4.

Two types of experiments were conducted in order to determine the position of the inhibition in the glycolytic mechanism (table 6). Glycogen

TABLE 5

Glycolysis with varying amounts of mercuric chloride and p-chloromercuribenzoic acid

EXPERIMENT	DATE	GLYCOLYSIS: CUBIC MILLIMETERS CO_2 IN 30 MINUTES *Milliliter mercury compound solution*							
		0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.40
	Jan.								
1	6	112	123	77		38		18	
2	7	134	94	36		17		2	
3	26	262		56		23		23	30
	Feb.								
4	5	204		123		71		14	17
5	6	270		244		185			58
6	8	217			172	111	75		

Concentrations of extract, sodium bicarbonate and glycogen were the same as given in table 2.

Experiments 1 and 2. p-Chloromercuribenzoic acid, 20 mgm., was shaken with 5.6 ml. of 0.01 N sodium hydroxide. The supernatant fluid was used. The residue was dried and weighed. In experiment 1, 7.0 mgm. and in experiment 2, 9.6 mgm. was in solution.

Experiment 3. $HgCl_2$, 0.1 per cent solution.

Experiment 4. $HgCl_2$, 0.05 per cent solution.

Experiments 5 and 6. $HgCl_2$, 0.025 per cent solution.

determinations were made on the extracts with and without phenylmercuric hydroxide to ascertain whether the glycogen disappeared from these extracts. Not a trace of glycogen was found in either the normal or the mercurial treated enzyme solutions after they had been shaken for two hours at 25°. However, determinations of the "total carbohydrate" in the mixtures immediately after and several hours after the addition of the mercury compound revealed no large change. In untreated controls a decrease of five or more milligrams in "total carbohydrate" was observed. From these two sets of experiments it appears that all of the glycogen may be converted into reducing substances in the presence of the mercury

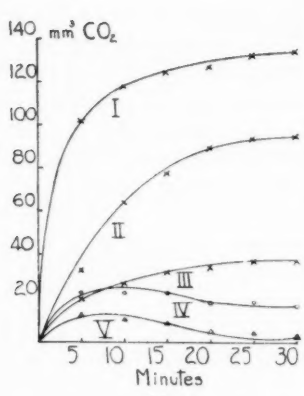

Fig. 3

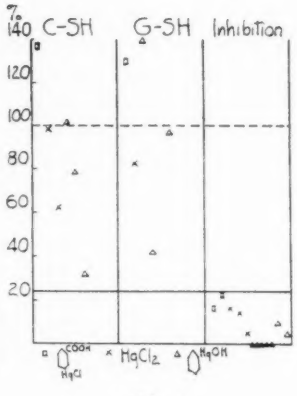

Fig. 4

Fig. 3. Inhibition of glycolysis with varying amounts of 0.186 per cent p-chloromercuribenzoic acid solution. Curve I, normal; curve II, 0.05 ml.; curve III, 0.1 ml.; curve IV, 0.2 ml.; curve V, 0.3 ml.

Fig. 4. Inhibition of glycolysis with various mercury compounds and restoration with cysteine and glutathione. Results are given in terms of per cent of glycolysis of normal extracts. C–SH indicates addition of cysteine hydrochloride after inhibitor; G–SH, glutathione.

TABLE 6

Glycogen and "total carbohydrate" determinations in muscle extract with and without phenylmercuric hydroxide

EXPERIMENT	DATE: JANUARY 1937	TIME OF EXPERIMENT	ANALYSIS G = GLYCOGEN T.C. = TOTAL CARBOHYDRATE	BEGINNING *"milligram glucose"*	END *"milligram glucose"*	
					Without mercurial	With mercurial
		hours				
1	18	2	G	7.68	None	None
2	19	2	G	11.00	None	None
3	20	2	T.C.	16.5	10.5	15.4
4	21	3	T.C.	15.6	10.2	14.9
5	22	2	T.C.	17.4	12.1	17.6

Solutions used: Extract, 3.0 ml.; sodium bicarbonate, 0.8 ml. of 1.3 per cent solution; glycogen, 0.2 ml. of 4.0 per cent solution; phosphate, 0.2 ml. of a solution of 0.1375 per cent $NaH_2PO_4 \cdot H_2O$ and 0.635 per cent of Na_2HPO_4; water, 0.2 ml. Phenylmercuric hydroxide, 30 mgm. shaken with 10 cc. of the extract. Centrifuged. 3.0 ml. used. Total volume in each vessel, 4.4 ml.

compound; and, on the other hand, only a very small portion (or none) of the reducing substances disappears.

Reversible inactivation with iodine. A series of determinations were made manometrically in which iodine was used as the inhibitor and

reduced glutathione, cysteine hydrochloride or ascorbic acid as reactivators (figs. 5 and 6). The inhibition with iodine was practically complete except in two experiments, in which, however, partial inhibition was observed. Reactivation with cysteine, glutathione or ascorbic acid was variable. In two experiments with cysteine, reactivation represented almost 100 per cent of the normal value while in some of the experiments in this series no reactivation occurred.

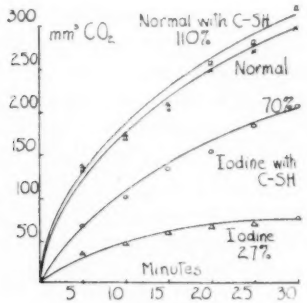

Fig. 5

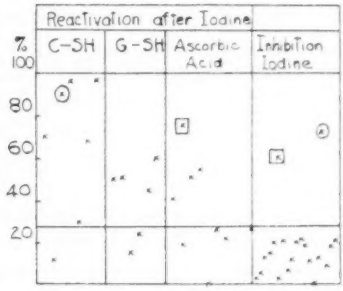

Fig. 6

Fig. 5. Inhibition of glycolysis with iodine and restoration with cysteine. Muscle extract, 1.2 ml.; 0.4 ml. of 1.3 per cent sodium bicarbonate; 0.2 ml. of 0.01 N iodine; 0.1 ml. of 10 per cent glycogen; 0.1 ml. of 3 per cent cysteine hydrochloride. In the vessels containing cysteine hydrochloride, there was added extra sodium bicarbonate equivalent to the acidic reagent.

Fig. 6. Summary of experiments with iodine as the inhibitor and cysteine, glutathione and ascorbic acid as reactivants. Results given in terms of per cent of glycolysis of normal solutions. Concentrations of solutions: 1.2 ml. of muscle extract; 0.4 ml. of sodium bicarbonate; 0.2 ml. of 0.01 N or 0.012 N iodine, 0.1 ml. of 10 per cent glycogen; 0.1 ml. of 3 per cent or 6 per cent cysteine hydrochloride; 0.2 ml. of 10 per cent glutathione; 0.2 ml. of 5 per cent ascorbic acid. In the vessels containing cysteine hydrochloride, glutathione or ascorbic acid there was added extra sodium bicarbonate equivalent to the acidic reagents. The pairs of points enclosed by circles and by squares identify partial inhibitions which were reversed by the action of cysteine and ascorbic acid, respectively.

Since the several reaction mixtures used necessarily varied in their content of total organic matter capable of reacting with iodine, the difficulty of avoiding an irreversible inactivation of labile catalysts by the addition of an excess of the reagent in some of the experiments is obvious. In a few of the experiments, the ratio between the amount of iodine used for inhibition and the total amount of iodine which the extract was able to absorb was obtained by suitable titration. The ratios were 0.21, 0.24, 0.26, 0.25, 0.22, averaging 0.24. Thus only 24 per cent of the total capacity of the extract for combining with and reducing iodine was used; of this only a fraction presumably reacted with enzymes.

Leövey (1936) working in these laboratories, has shown that the conversion of pyruvate to lactate in rabbit muscle pulp is inhibited by iodine and quinone and restored by H_2S.

Discussion. Although the inhibitory action of mercury compounds and of iodine upon glycolysis cannot be fully interpreted at present, the action of the organic mercury compounds used in this work might be attributable to a direct combination of the reagent with a specific group of an enzyme molecule; that of iodine to the oxidation of such a group. It is tempting to consider the observations in terms of reversible chemical interactions with a sulfhydryl group of an enzyme molecule. The effective mercury compounds are known to form mercaptides with authentic sulfhydryl compounds; such mercaptans are known to be regenerated by the action of the reactivators used in this investigation. Furthermore, iodine, under proper conditions, oxidizes mercaptans to the corresponding dithio compounds; the latter are readily reduced to the parent mercaptans. There is provided a plausible explanation for the complete inhibition of glycolysis by adequate amounts of the mercury compounds and for the relationship observed between varying amounts of these compounds and the extent of glycolysis.

It must be emphasized, however, that the evidence is circumstantial at present. Under certain conditions mercury compounds like those used in these experiments react with amino or other groups characteristic of proteins. A coenzyme may possibly be involved in the inhibition of activity. This would not be glutathione, functioning as coenzyme for methylglyoxalase, since Lohmann (1932) has shown that glycolysis may proceed without this substance. It is unlikely that mercury compounds function as accelerants of oxidation of the enzyme; such an acceleration of oxidation might, however, account in part for the inhibitory effect of copper ion on glycolysis as observed by Lipmann (1934) and Wagner-Jauregg and Rzeppa (1936). Lipmann (1929) has presented evidence that the inhibitory action of the fluoride ion upon glycolysis may involve a combination of the fluoride with a heavy metal component in an enzyme molecule; on this basis the inhibitory action of oxidizing agents might be related to the oxidation of the metallic component.

Although the sulfhydryl hypothesis seems to provide a more plausible explanation for the reversible inhibition of glycolysis by mercury compounds and oxidizing agents as observed here, a final answer will be obtained only after isolation and purification of the enzymes concerned in the inhibition.

SUMMARY

In small concentrations, phenylmercuric hydroxide, p-chloromercuribenzoic acid and mercuric chloride inhibit glycolysis in extracts of frogs'

muscle. This inhibition may be abolished by the use of cysteine or glutathione. The degree of inhibition depends on the concentration of the mercury compound. A block in the glycolytic mechanism occurs probably at the hexose stage. An inhibition produced by iodine is also shown to be reversible, the reactivants used being cysteine, glutathione and ascorbic acid. This is tentatively interpreted in terms of an oxidative-reductive control of catalysis of the glycolytic process in frogs' muscle.

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SOME EFFECTS OF INTRAPERITONEAL GLUCOSE INJECTIONS AND EXCESS WATER IN NORMAL, ADRENALECTOMIZED, AND HYPOPHYSECTOMIZED RATS

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Schechter et al. (1933) and Darrow and Yannet (1935, 1936) showed that the intraperitoneal injection of an isotonic solution of glucose would cause a shift of part of the body electrolytes into the peritoneal cavity. This reduction in osmotically active components of body fluid caused in turn a transfer of water into cells and transiently into the peritoneal cavity with a resulting hemoconcentration. The changes induced by glucose injections, as pointed out by Gilman (1934), are somewhat analogous to the effects of adrenalectomy; and the technique has been used in studies of adrenal problems (Swingle, Parkins and Taylor, 1936; Harrop, 1936; Robinson and Hegnauer, 1936). The above workers have generally left the injected solution in the peritoneal cavity for about six hours and then removed it by paracentesis. This depleted their animals of the electrolyte which had been transferred to the ascitic fluid.

Using this technique, we have studied some of the varying initial responses of normal, adrenalectomized, and hypophysectomized rats to glucose injections and the final disposition of the injected fluid when it remained in the peritoneal cavity for a twelve hour period. The symptoms resulting from this treatment suggested certain similarities to the effects reported for intoxicating doses of water, and we have therefore included here some comparable studies on water intoxication.

In these studies only the fate of the fluid administered has been considered. One of the authors is measuring the concomitant electrolyte shifts. Water shifts were determined first because, in the rat, where repeated serum electrolyte determinations are difficult, the behavior of water gives definite clues as to where and when to anticipate the significant electrolyte changes.

METHODS. Thirty-two normal, 44 adrenalectomized and 36 hypophysectomized animals were studied after glucose injections. The rats were mature and of both sexes. They were deprived of food but not water for twelve hours before the experiments were started. Adrenalectomy was performed from 18 to 24 hours before glucose injection or water intoxica-

tion, a time chosen because the animals had recovered from the effects of the surgery and no apparent symptoms of adrenal insufficiency had yet developed. Hypophysectomy was done from 2 to 20 days before experiments were run and the response seemed to be independent of that interval. Hypophysectomized animals were given sugar water to drink for several days after the operation. The completeness of hypophysectomy was determined by gross observation of the sella contents, body weight loss, and reduction of adrenal size.

A dose of 0.1 cc. per gram body weight of 5.5 per cent glucose, warmed to body temperature, and injected intraperitoneally, was used. To

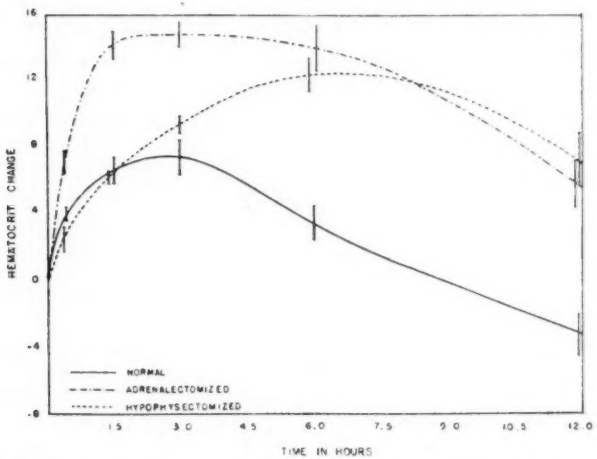

Fig. 1. A graph showing mean hematocrit changes following intraperitoneal glucose injections. Twenty-six normal, 32 adrenalectomized and 23 hypophysectomized animals were used. The vertical lines represent the probable error at the points indicated. Half-hourly determinations were made for the first three hours and at 1½ hour intervals thereafter.

prevent leakage, the punctures through the skin and abdominal muscle wall were made at slightly different levels. The animals were kept in metabolism cages for the collection of urine. As an index of hemoconcentration, hematocrit readings (quadruplet samples in capillary tubes) were made at intervals of a half hour or longer. When the blood was free-flowing, tail blood samples were used, otherwise the femoral vein was tapped. Initially it was attempted to make parallel hematocrit, hemoglobin, and red cell count readings, but in animals in which a high hemoconcentration had been induced, blood flow was so sluggish that this was usually impossible.

At the end of the twelve hour period the animals were lightly anes-

thetized and any ascitic fluid removed by suction through an abdominal incision.

Response to glucose injections. 1. *Hemoconcentration.* a. In response to glucose injections normal animals showed a rise in hematocrit within one-half hour. This rise approached or reached its maximum at 1.5 hours. From 1.5 to 3 hours the hematocrit remained stationary or rose slightly (fig. 1).

b. The response of adrenalectomized animals occurred in approximately the same time, but the hematocrit rise was at least twice as great as in normal rats (fig. 1). Dark, viscous, slow-flowing blood also indicated a high hemoconcentration.

TABLE 1

Mean results of intraperitoneal glucose injections in normal, adrenalectomized and hypophysectomized rats, with paracentesis at 12 hours

	NORMAL	ADRENALECTOMIZED	HYPOPHYSECTOMIZED
1. Number of animals used	17	17	13
2. Number of animals re-diluting blood to normal levels	16 (94%)	5 (29%)	1 (8%)
3. Number of animals secreting urine	16 (94%)	6 (35%)	1 (8%)
4. Per cent injected fluid recovered as urine	43.75%	3.33%	0.03%
5. Per cent injected fluid recovered by paracentesis	30.00	41.90	94.00
6. Number of rats absorbing all injected fluid	6 (37.5%)	1 (6%)	0
7. Number of rats absorbing no injected fluid	0	0	7 (54%)
8. Per cent injected fluid recovered (urine + ascitic)	73.75	45.23	94.03
9. Per cent change in body weight	−3.95	+1.38	−1.53
10. Number that died during experiment	0	6 (35%)	6 (46%)

c. Hypophysectomized rats showed a definite, although variable, refractoriness to the treatment during the initial stages with a slower response throughout. They eventually reached as high a hematocrit level as that in adrenalectomized animals, but did so at about six hours in contrast to 1.5 hours for adrenalectomized rats (fig. 1).

2. *Hemodilution* (see table 1, no. 2; and fig. 1). a. Normal animals began to redilute their blood at about 3 hours, and in most cases their hematocrit indicated a less-than-normal level before 12 hours.

b. Adrenalectomized rats tended to hold their hemoconcentration for long periods. Two-thirds did not return to normal hematocrit levels, and in all cases dilution was delayed. Those that died during the experiment were uniformly in the group which failed to redilute.

c. Hypophysectomized animals, with only one exception, failed entirely to dilute their blood to normal levels within 12 hours.

3. *Urine flow* (see table 1, nos. 3 and 4). a. All but one normal animal secreted urine during the experiment, and on the average 43.75 per cent of the injected fluid was thus eliminated within 12 hours. The time of first urine flow could be predicted by hematocrit readings, as urine was never secreted until the hematocrit returned to approximately normal ranges. As a sub-normal dilution was reached urine flow increased.

b. Only one-third of the adrenalectomized animals secreted urine during the 12 hour period and an average of only 3.33 per cent of the injected fluid was thus eliminated. That this was not due to a renal failure is indicated by the fact that whenever hemoconcentration was relieved, urine flow did occur.

TABLE 2

Effect of glucose injections and paracentesis at 3 hours

	GLUCOSE SOLUTION INJECTED	ASCITIC FLUID RECOVERED	CHANGE IN VOLUME OF ASCITIC FLUID	CHANGE IN VOLUME OF ASCITIC FLUID	WEIGHT CHANGE	SYMPTOMS
	cc.	*cc.*	*cc.*	*per cent*	*per cent*	
Mean of 6 normal rats	19	25.2	+6.2	+32.6	−5.26	Lethargy
Mean of 8 adrenalectomized rats	16.6	16.0	−0.6	−3.6	−1.32	All died within 24 hours
Mean of 6 hypophysectomized rats	15.2	19.1	+3.9	+25.7	−5.59	All died within 24 hours

c. Hypophysectomized animals, concentrating more slowly, put out, as a group, practically no urine in the 12 hour period which again was correlated with a retained hemoconcentration.

4. *Transfer of fluid to peritoneum* (see table 2). Schechter and others pointed out that the initial shift of electrolyte into the ascitic fluid after glucose injections resulted also in a shift of some water into the peritoneal cavity, i.e., at certain stages there was more peritoneal fluid than was injected. Since our adrenalectomized animals, within three hours, showed a greatly elevated hematocrit, as compared to normal rats, we considered it probable that this greater loss of blood fluid was in part due to an augmented shift of water into the peritoneal cavity. When, however, a group of adrenalectomized rats were given glucose injections and paracentesis performed at 3 hours, less fluid was present than in either normal or hypophysectomized animals.

a. Normal animals, 3 hours after glucose injections, had invariably

transferred considerable body fluid to the peritoneal cavity, a mean of 32.6 per cent more fluid being present than had been injected.

b. Adrenalectomized animals, on the other hand, had either transferred only small amounts of fluid into the peritoneal cavity or had actually absorbed part of the injected fluid at three hours. The average result for adrenalectomized rats was an absorption of 3.6 per cent of the injected fluid, as contrasted to a shift of 32.6 per cent in the opposite direction in normal animals.

c. Hypophysectomized animals behaved essentially like normal ones and augmented their injected ascitic fluid by 25.7 per cent at 3 hours.

5. *Absorption of fluid by twelve hours* (see table 1, nos. 5, 6, and 7). a. At the end of the 12-hour period normal rats had absorbed 70 per cent of the injected fluid. Actually much more fluid than this was absorbed due to the initial outward shift as discussed above. All normal rats absorbed at least part of the fluid, and more than one-third absorbed all of it.

b. In adrenalectomized rats, although removal of peritoneal fluid began earlier, and the initial shift of fluid toward the peritoneum was lacking, the average result was a total absorption of 58.1 per cent of the injected fluid. Some fluid was absorbed in every case. Animals which survived the experiment absorbed approximately the same amounts as did normal rats, while those which succumbed absorbed lesser amounts.

c. The primary difference between hypophysectomized rats and the other two groups was in the amount of fluid absorbed from the peritoneal cavity. The hypophysectomized rats had absorbed, at 12 hours, an average of only 6 per cent of the injected fluid. Fifty-four per cent of them either absorbed no fluid or else increased the volume of ascitic fluid. This retention of fluid in the peritoneum was believed responsible for the prolonged hemoconcentration observed in these animals. The reason for the non-absorption of fluid was probably an inhibited ability to absorb glucose, rather than any peculiarity in electrolyte behavior. Phillips and Robb (1934), Bennet (1936) and Samuels and Ball (1937) have shown that glucose is not absorbed at a normal rate from the intestine after hypophysectomy, a deficiency which occurs quickly after operation. Samuels and Ball, on the basis of blood sugar determinations, concluded that there was no such inhibited absorption of glucose from the peritoneal cavity after hypophysectomy. They failed to take into account, however, that the injection of intraperitoneal glucose perhaps induced a considerable hemoconcentration thus giving an abnormal elevation to their sugar curves.

6. *Fate of absorbed fluid* (see table 1, nos. 8 and 9). a. At 12 hours normal animals retained in the peritoneal cavity 30 per cent of the fluid injected. Approximately 44 per cent of the amount injected was recovered as urine, leaving 26 per cent unaccounted for. Part of this

missing fluid was in the blood stream (and presumably also in interstitial fluid), as indicated by subnormal hematocrit levels.

b. In adrenalectomized animals, 42 per cent of the injected fluid was in the peritoneal cavity at 12 hours, but only 3.33 per cent was recovered as urine. Thus, 55 per cent of the injected fluid was unaccounted for. This fluid, unlike that in the normal animal, was not in the blood stream (nor, presumably, in the interstitial spaces), as indicated by the continued hemoconcentration. It must have been shifted, therefore, into cells. That the fluid was actually retained at some extra-vascular site was further indicated by the appearance of puffiness, some symptoms of water intoxication, and the fact that after paracentesis the adrenalectomized rats had gained on the average 1.38 per cent of their original body weight, while normal animals similarly treated had lost 3.95 per cent of their original weight.

This tendency to shift all available fluid toward the tissues, in amounts greatly in excess of normal animals, is the most significant feature (among those which we have observed) of the response of the adrenalectomized animal to glucose injections.

c. In the hypophysectomized animals, as mentioned above, nearly all of the fluid remained in the peritoneal cavity.

7. *Controls.* The responses found here for operated animals were not due to the effects of surgery or trauma as indicated by the normal reactions of rats either incompletely hypophysectomized or with small parts of the adrenals removed.

Effect of cortical extract on the response of glucose-injected, adrenalectomized and hypophysectomized rats. Four adrenalectomized rats were given 2 cc. of cortical extract[1] on the day of operation, and the following day glucose experiments were run. During the experiment 3.75 cc. of cortical extract were given in divided doses. (The cortical extract used here was made up in 0.9 per cent NaCl and probably the salt in itself was of some remedial value.)

The cortical extract almost completely eliminated the characteristic responses of adrenalectomized rats to glucose injections (table 3). The initial hematocrit rise was high but, as in normal animals, quickly returned to normal values. Correlated with the early hemodilution, urine flow began at about 5 hours as in normal animals. However, as in untreated adrenalectomized rats, there was a considerable absorption of fluid by tissues, as indicated by the fact that nearly half of the fluid could not be accounted for at 12 hours either by ascitic fluid or urine, and also by the fact that the body weight loss, characteristic of normal animals, was

[1] The authors are indebted to Dr. G. F. Cartland of the Upjohn Company for kindly supplying the cortical extract used in these experiments.

missing. The general resistance to the glucose injections was greatly improved, however, as none of the animals showed ill effects during, or for several days following the experiment.

Four hypophysectomized animals, operated for 5 days and treated after operation with 0.75 cc. cortical extract daily (a dose sufficient to prevent all symptoms of adrenal insufficiency in adrenalectomized rats) were likewise given glucose injections on the sixth day. During the experiment, 3.75 cc. of cortical extract were injected. The results were variable, and it is, therefore, impossible with this small number to determine whether the hormone assisted in fluid disposal (table 3). Two responded after

TABLE 3

Effect of intraperitoneal glucose in adrenalectomized and hypophysectomized rats receiving cortical hormone with paracentesis at 12 hours

	ADRENALECTOMIZED				HYPOPHYSECTOMIZED			
Animal number	V	VI	VII	VIII	I	II	III	IV
Maximum rise hematocrit	10.90	9.27	14.00	14.75	10.10	10.09	3.03	7.49
Time of re-dilution of blood (hours)	5	5	5	6.5	None	7	11	4
Time of first urine flow (hours)	5	5	5	6.0	None	5.5	8	3.5
Per cent injected fluid recovered as urine	44.0	60.0	67.0	35.0	0	62.0	42.0	71.0
Per cent injected fluid recovered by paracentesis	0.0	0.0	0.0	19.0	83.0	17.0	58.0	5.0
Per cent injected fluid recovered (urine + ascitic)	44.0	60.0	67.0	54.0	83.0	79.0	100.0	76.0
Per cent change in body weight	+0.55	−0.65	−0.58	+0.69	−3.28	−2.89	−6.84	−3.03

this treatment like normal animals, and two like untreated hypophysectomized animals (i.e., showed an inability to absorb the peritoneal glucose solution). The cortical extract was certainly helpful in the general resistance to the effects of the experiment, as practically no signs of lethargy and weakness, generally characterizing the response, were apparent and all animals lived for long periods after the experiment.

Water intoxication. In the course of the glucose injection experiments, we frequently noticed, during the periods when the peritoneal fluids were being absorbed, a hyperexcitability, twitchiness, and, upon handling, an ophisthotonus reflex. This occurred particularly in the adrenalectomized rats, and suggested a similarity to the effects of water-intoxication, to which Swingle, Parkins, Taylor, and Hays (in press) have shown that

adrenalectomized dogs are exceedingly sensitive. Rigler (1935) has reported a similar finding for adrenalectomized mice. We tried therefore to determine whether excess fluid given by mouth would produce effects similar to those resulting from glucose injections.

Water intoxication as described for the rat and other forms by Rowntree (1926) can be produced in fasted normal animals by administering .09 cc. of water per gram body weight via stomach tube at six hourly intervals. The symptoms are marked first by a lethargy, then occasional twitchy movements, and finally a series of convulsions, one feature of which is oftentimes a striking ophisthotonus with the tail uplifted at right angles to the body.

We administered excess water to 16 normal, 7 adrenalectomized and 16 hypophysectomized rats, of which several typical cases are shown in table 4. The standard amounts of water given were five hourly doses of .06 cc. warmed to body temperature, per gram body weight. This dosage never produced an intoxication, as judged by collapse and convulsions, in normal animals, but will consistently do so in adrenalectomized or hypophysectomized animals.

In contrast to the responses following glucose injections, adrenalectomized and hypophysectomized animals behave similarly to excess water by mouth. They were thrown into collapse or convulsions almost invariably within 4 to 7 hours, and with rare exceptions died within 24 hours. They began secreting urine at the same time as normal rats (about one hour after the first water was given) but this secretion was always slight and at 8 hours from the beginning of the experiment only a small fraction of the fluid given had been excreted. Within 8 hours normal rats, on the other hand, had excreted about 85 per cent of the fluid administered.

Within 24 hours, the few operated animals still living retained large amounts of fluid, as indicated by continued convulsions, weight increase, and scant urination. It is not clear in these experiments, as it was after glucose injections, that hemoconcentration was the cause of slight urine flow. The factors involved are being studied further.

Water intoxication in cortical extract-treated, hypophysectomized rats. Due to the similarity in susceptibility to water intoxication after adrenalectomy or hypophysectomy, we thought it of interest to determine whether adrenal cortical extract would remove the deficiencies in hypophysectomized rats. After hypophysectomy, cortical extract was given from the time of operation, and on the day of the experiment 4 cc. were injected in four doses. This treatment, as is evident from table 4, sec. IV, almost completely restored all hypophysectomized animals to normal as judged by gross symptoms and urine flow.

This remedial effect was, however, in sizable part, due to the NaCl contained in the extracts, as is evident from the response of hypophysec-

TABLE 4

Water intoxication in normal, adrenalectomized and hypophysectomized rats
(Dosage: 5 hourly doses of .06 cc. H_2O/gram body weight)

INDEX	TOTAL DOSAGE*	URINE AT 8 HOURS	URINE AT 24 HOURS	FLUID EXCRETED 24 HOURS	WEIGHT CHANGE 24 HOURS	SYMPTOMS
			Sec. I. Normal rats—Untreated			
	cc.	*cc.*	*cc.*	*per cent*	*grams*	
4/3-V	46.0	38.1	40.2	87	−16	Twitchy
4/3-VI	49.5	38.2	40.6	82	−9	Lethargy
			Sec. II. Adrenalectomized—Untreated			
4/3-I	52.5	8.0	16.3	31	+15	Convulsions, 7 hours; survived
4/3-II	48.0	2.2	2.2			Convulsions and death, 5 hours
4/3-III	46.5	2.2	2.2	4.7	+27	Convulsions, 5 hours; died, 24 hours
			Sec. III. Hypophysectomized—Untreated			
4/3-VIII	43.5	2.1				Convulsions, 5.5 hours; died, 8 hours
4/3-IX	52.5	7.8				Convulsions, 4 hours; died, 8 hours
4/3-X	45.0	10.2	17.2	38.2	+3	Convulsions, 5 hours; died, 24 hours
			Sec. IV. Hypophysectomized—Cortical extract treated (4 cc.)			
4/10-II	40.0	36.4	40.1	91.1	−6	Twitchy; survived
4/10-III	43.4	35.9	45.3	95.8	−8	Lethargy; survived
4/10-IV	65.0	44.8	59.4	86.1	−9	Lethargy; survived
4/21-I	54.0	24.4	29.0	53.7	+9	Convulsions, 5 hours; recovered, 7 hours
			Sec. V. Normal—Normal saline treated (0.08 cc. normal saline/cc. H_2O)			
4/10-V	50.0	52.8	54.8	101.5	−17	Twitchy
4/10-VI	65.0	59.3	54.8	79.4	−21	None
4/21-X	43.5	37.7	40.9	94.0	−8	None
			Sec. VI. Hypophysectomized—Normal saline treated (0.08 cc. normal saline/cc. H_2O)			
4/21-II	54.0	13.1	21.6	40.0	+11	Convulsions, 4 hours; survived
4/21-III	64.5	29.7	48.6	75.3	−9	Twitchy; survived
4/21-VI	43.5	11.5	12.5	28.7	+10	Convulsions, 5 hours; died, 36 hours
4/22-VII	64.8	7.6	7.6	11.7	+17	Convulsions, 5 hours; died, 40 hours

* Total dosage includes water given by stomach tube plus volume of normal saline or cortical extract injected.

tomized rats treated with normal saline alone (table 4, sec. VI). However, a comparison of sections IV and VI of table 4, will indicate that 0.9 per cent NaCl alone is not as effective as the cortical extract in protecting hypophysectomized rats from excess water. This indicates that the damaged adrenal of the hypophysectomized rat is an important factor in its susceptibility to water intoxication.

That saline is helpful in the relief of water intoxication symptoms in normal animals is well known from the work of Rowntree and that small doses of saline assist in the elimination of sub-intoxicating doses of water in normal rats was clearly shown in our experiments. (Compare sec. I and sec. V of table 4.)

Discussion and conclusions. The early hemoconcentration, loss of sodium salts from the blood, and rise of serum potassium after adrenal removal are factors around which several current theories of adrenal cortical function revolve. It is not clear that electrolyte changes, of a type compatible with current ideas of electrolyte partitioning, will explain the changes in water distribution. That changes in renal function alone will explain completely the mal-distribution of fluid and electrolyte is likewise not well established (Swingle et al., 1936; Ingle et al., 1936). The fact that hemoconcentration is not entirely due to a renal loss of fluid but partly to an internal shift seems to be demonstrated (Swingle et al., 1934, 1936; Harrop, 1936). Such internal shifts of water are apparently characteristic of an animal lacking adrenal glands, as indicated in the experiments reported here where the phenomenon was placed in an exaggerated light and where, in the glucose experiments, the kidney was almost completely excluded from the picture. Whether internal electrolyte changes can be satisfactorily correlated with the anomalous water shifts we have yet to determine.

The differences in the reaction to intraperitoneal glucose injections of normal intact animals and animals in apparent normal health from which the adrenal glands have just been removed may be summarized as follows:

a. The adrenalectomized animal after glucose injection concentrated its blood to a level approximately twice that of a normal animal.

b. The adrenalectomized rat attained this initial high hemoconcentration almost entirely by shifting fluid out of the blood stream toward the tissues; and not, as in the normal rat, by shifting the fluid in part into the peritoneal cavity.

c. Fluid absorbed from the peritoneal cavity was not used (quickly, at least) as in the normal animal, to re-dilute the blood but was shifted into the cells, resulting in prolonged periods of hemoconcentration. The assumption that this fluid goes into cells and not into the interstitial spaces is based upon work indicating that in normal animals the dilution of an ultra-filtrate of blood and interstitial fluid is essentially similar (Peters,

1935); and, on the work of Harrop, 1936, who showed that both in adrenal insufficiency and after intraperitoneal glucose injections in normal dogs there is a loss of both interstitial and vascular fluid. Changes induced, therefore, in vascular and interstitial fluid are probably similar. That the fluid was retained at some extravascular site was indicated by weight gains after paracentesis, whereas normal animals invariably lost weight. That the fluid is intracellular is further indicated by symptoms of water intoxication.

d. This absorption by cells of the fluid taken up from the peritoneal cavity, making the water unavailable for secretion, is probably sufficient to account for the anuria. Renal function, at least as far as the elimination of water is concerned, seems unimpaired.

e. The tendency of the adrenalectomized animal to transfer absorbed fluid to tissues was so strong that it could not be completely eliminated even by large doses of cortical extract in saline solution.

The inability of the hypophysectomized rat to absorb injected fluid (probably because of impaired ability to absorb glucose itself) prevented analyses comparable to the above within the time limits of these experiments. The refractoriness to hematocrit change in the hypophysectomized animals is without adequate explanation.

When adrenalectomized animals are given excess water by mouth, the absorbed fluid is likewise quickly shifted to cells as indicated by the rapid appearance of symptoms of water intoxication.

The hypophysectomized rats were as susceptible to water intoxication as were those without adrenals and this deficiency is probably due, in large part, to the damaged adrenal cortex. The possible differential involvements of different parts of the hypophysis in this response are not considered here, because all animals on which conclusions were based were apparently completely hypophysectomized.

SUMMARY

1. The effects of injections of isotonic glucose solution into the peritoneal cavities of normal, adrenalectomized, and hypophysectomized rats were studied. At 12 hours, the peritoneal cavity was drained of any remaining fluid.

2. Adrenalectomized rats responded to intraperitoneal glucose injections by an initial rise in hematocrit about twice that in normal rats, by a prolonged hemoconcentration even during the period when the glucose solution was being absorbed, and by an almost complete anuria.

3. The hemoconcentration of adrenalectomized rats, following glucose injections, unlike that in the normal animal, was due entirely to a shift of fluid from the blood stream toward the tissues. The expected shift of body fluid to the peritoneal cavity was generally lacking.

4. The fluid absorbed from the peritoneal cavity in the adrenalectomized rat was not used, initially at least, as in normal rats, to re-dilute the blood, but was shifted to extra-vascular depots, presumably into cells.

5. Adrenalectomized rats were largely, but not completely, protected from the effects of glucose injections by large amounts of cortical extract in saline solution.

6. Hypophysectomized animals differ greatly from either normal or adrenalectomized rats in their response to glucose injections in that they showed a delayed hematocrit change and were unable, at least within 12 hours, to remove sizable quantities of fluid from the peritoneal cavity. This was probably due to an impaired ability to absorb glucose.

7. Both adrenalectomized and hypophysectomized rats were susceptible to water intoxication, and succumbed to doses having little deleterious effect upon normal animals.

8. This susceptibility was due to the fact that, as in adrenalectomized animals given glucose injections, most of the absorbed fluid was shifted into tissues and not excreted as in normal animals.

9. The susceptibility of hypophysectomized animals to water intoxication was probably largely due to adrenal cortex damage, as the deficiencies could be remedied completely by cortical extracts in saline solution, and the benefit could not be completely explained by the salt in the extract.

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THE RÔLE OF THE CEREBRAL CORTEX AND OF VARIOUS SENSE ORGANS IN THE EXCITATION AND EXECUTION OF MATING ACTIVITY IN THE RABBIT[1]

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Copulation and the associated mating behavior, although very primitive and essential in character, require a delicate coördination of muscular activity. It is obvious that these activities constitute a reaction pattern which is essentially a form of emotional behavior. Elicitation of such behavior under specific conditions must depend upon stimuli received by olfactory, auditory, visual, tactile or some other group of afferent endings. It is conceivable that the relative importance of each of these various sensory stimuli might vary from time to time or might be changed by the removal of other organs of special sense. Loss of certain types of sensation due to cortical ablation might likewise increase the importance of other kinds of stimuli. Stone in 1925 demonstrated that removal of the olfactory bulbs and portions of the frontal half of the neocortex of male rabbits in no way interfered with their sexual activity. Bard (1934) observed treading, rolling, and other signs of sexual excitement in a female cat from which the neocortex had been completely removed. This animal also mated and exhibited all the indications of heat. Except for this work and other studies by Bard (Bard, 1936; Bard and Rioch, 1937) very little has been done to ascertain to what extent sexual behavior of mammals is dependent upon the integrity of various parts of the cerebral cortex and subcortical structures.

In the work here described rabbits were studied. This species is of peculiar interest in that ovulation normally occurs only on coitus or as a result of strong sexual excitation. Thus the occurrence of ovulation furnishes an additional indication of the normality of behavior and a means of judging the effectiveness or intensity of emotional excitement. A minor advantage is that the female rabbit is relatively constantly receptive and males are invariably active sexually.

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The sexual behavior of male and female rabbits has been described by numerous workers (Hammond, 1925). The male mounts almost immediately when the female is placed with him. If the female is receptive intromission occurs very quickly and the male falls off, presumably when he ejaculates, usually uttering a characteristic cry as he does so. After falling off the male hops about for a few minutes stamping with his hind feet upon the floor of the cage and then mounts again. The female does not exhibit such a complex type of response. If receptive, she responds when mounted, chiefly by raising her tail and hind parts. Failure of the female to react in this way prevents intromission. These are the reactions which will be spoken of as normal sexual behavior. Normality of mating behavior does not indicate that a female is capable of caring for and rearing young. This paper does not include a consideration of all the reproductive activities of the female.

METHODS. In testing the receptivity of females and the activity of males the female was always placed in the buck's cage. Mating generally occurs more promptly if that procedure is followed rather than the reverse. In cases in which there was any doubt as to the occurrence of intromission and ejaculation the presence or absence of sperm in a vaginal smear settled the question. Occasionally a female rabbit will receive a male and not ovulate. Young females will mate before ovulation is possible (Hammond, 1925) and adult females, though permitting intromission, frequently fail to ovulate when in poor physical condition. Much experience has shown, however that if a mature healthy female receives a male ovulation follows. In all the cases reported here sexual activity was not considered normal unless ovulation did occur subsequent to coitus. The occurrence of ovulation was determined by laparotomy and examination of the ovaries for corpora lutea four days after mating. During this interval the corpora lutea attain a degree of development which enables easy and unmistakable identification. Occasionally a female will mount another rabbit of the same sex, make copulatory movements, eventually fall off uttering a cry like that of a male and as a result ovulate. To prevent such activity all animals were kept in individual cages.

Under the rather artificial conditions existing in the laboratory, training and conditioning of the rabbits probably play some part in the elicitation of sexual activity. Females to be tested were invariably placed in the male's cage. Males accustomed to this practice mount cats, kittens, guinea pigs and even dead animals or inanimate objects which are placed with them. This nonspecificity of behavior may be due in part to their training. At any rate, all the males and females used were accustomed to this procedure before operation and the same method was employed in testing reactions to the opposite sex after operation .

Removal of certain cortical areas produces sensory deficiencies. Aboli-

tion of sexual activity as a result of a cortical ablation might therefore be due to a sensory loss, a motor deficiency, a loss of ability to translate a particular type of stimulation into an appropriate response or any combination of the three. An effort was made to ascertain the importance of pure sensory deficiencies uncomplicated by cortical insult. Vision was eliminated by enucleation of the eyes. Complete anosmia was easily produced by exposing and removing the olfactory bulbs through a small opening made in the rostro-dorsal aspect of the skull. An attempt was made to inactivate the auditory apparatus by destroying the labyrinths through openings in the bullae. For this an occipital approach (Camis, 1930) was used. Although hearing was apparently destroyed these operations failed to produce marked signs of labyrinthine deficiency. Absence of such deficiencies indicated that the labyrinths were only partially destroyed and that the ability to hear might have been impaired but not abolished. To make sure that no auditory sensibility remained these operations were supplemented by destruction of the middle ear. This complex was destroyed with a probe passed through the external auditory meatus. Since ovulation normally occurs only on coitus it has been suggested that stimulation of the genitalia and genital tract might be essential to this reaction. An estimation of the importance of this sensory factor was attempted by deafferentation of the pelvic region. This was done by removal of the lower segments of the lumbar and the entire sacral cord or by transection of the cord in the lumbo-sacral region. In addition the abdominal sympathetic chains were removed.

Brain operations were performed following exposure of the cortex by removal of the skull over the region to be ablated. Cortical tissue was carefully dissected away from the underlying structures with a small sharp tonsil dissector and an ear spoon. The moderate bleeding encountered was easily controlled by means of small cotton pledgets. The incision was closed by subcutaneous as well as skin sutures. Pentobarbital sodium (0.7 grain per kgm. of body weight) injected intraperitoneally was the anesthetic used; it was supplemented with ether when necessary.

The chief difficulty encountered was in maintaining the proper nutrition of those animals which were subjected to extensive cerebral ablations. Unless a rabbit begins to eat spontaneously within a week or two it is almost impossible to keep it alive and maintain its weight by feeding either with a stomach tube or by hand. This difficulty has thus far prevented the satisfactory study of chronic preparations which have undergone complete decortication plus extensive ablation of subcortical tissue. All animals ate normally if the cortical removal had not involved the olfactory tract. A few completely decorticate animals survived removal of the olfactory bulbs or severance of the olfactory tracts but they apparently could not find their food with ease and they did not eat enough spontaneously to preserve their normal body weights.

Following each operation the animals were studied and their ability to react tested before further operations were tried. Animals which failed to respond were kept for periods of a year to eighteen months and tested frequently before it was concluded that they would no longer mate.

RESULTS. In three males and two females the olfactory bulbs were removed without rendering these animals at all abnormal in their sexual activities. Enucleation of the eyes of three males and two females likewise failed to produce any deficiency. In two males and one female an essential part of the auditory apparatus was destroyed with the same lack of effect. Finally two males and two females were deprived of olfactory, visual and auditory end organs. These animals were as active sexually as before operation. It can be concluded that these three special senses are not essential for the elicitation of emotional excitement and sexual activity if the animals are otherwise normal. It follows, therefore, that any sexual inactivity resulting from cortical ablations cannot be referred solely to a loss of the cortical response to afferent impulses originating in nose, eye, or ear.

Six males and eight females were completely hemidecorticated with no resulting deficiencies in their ability to become sexually excited and sexually active. Two females were completely hemidecerebrated on the left side by unilateral removal of all central tissue lying ahead of the mesencephalon (fig. 1-D). The pituitary gland and some of its hypothalamic connections were left intact. The left optic tract was ablated but the chiasm and left optic nerve were not damaged. Two months were required for complete recovery but after that time they received males and ovulated normally. One male was prepared with a similar unilateral lesion and two others sustained operations in which not only the cortex but also the striatum and hippocampus of one side were removed. These three males showed normal sexual activity in spite of the unilateral motor deficiencies which rendered them more awkward than normal rabbits. It is safe to conclude 1, that both in the female and the male one-half of the forebrain can be removed without abolishing sexual activity, and 2, that the mere removal of a large amount of cerebral tissue does not in itself put an end to this form of behavior.

In several rabbits in which complete hemidecortication had been carried out various cortical areas of the remaining hemisphere were extirpated. Two males and one female sustained complete removal of the cortex of the left hemisphere and ablation of the occipital half of the right neocortex (fig. 1-B). These animals mated normally with one another as well as with normal rabbits of the opposite sex. Subsequent removal of the olfactory bulbs and enucleation of the eyes did not abolish their activity. One male survived a still more extensive cortical removal, namely, ablation of the entire left cortex, the occipital half of the right neocortex and most of

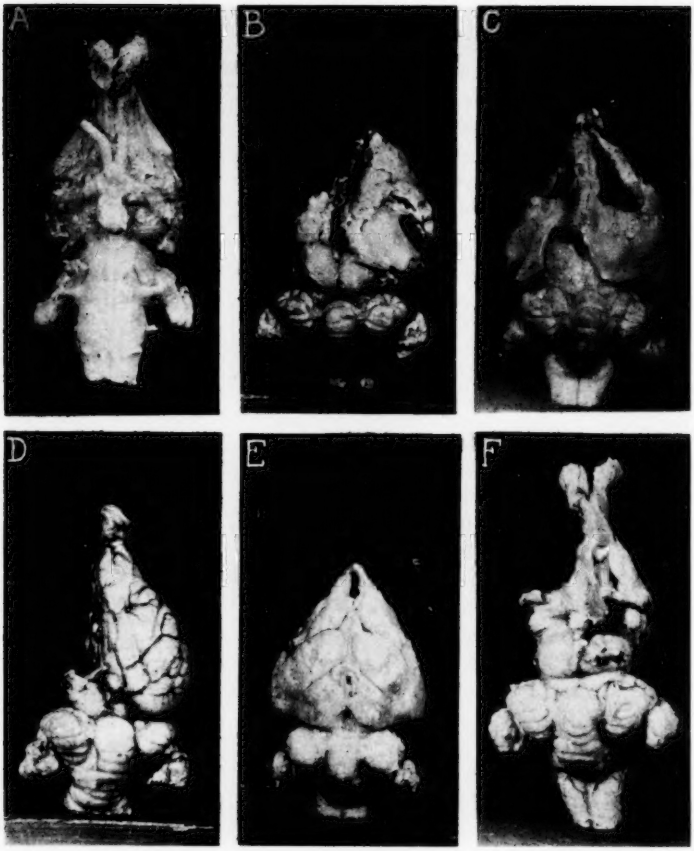

Fig. 1. A. Bilateral pyriform lobe removal in a female rabbit. Ventral aspect of brain. This animal mated and ovulated.

B. Brain of male rabbit which mated after ablation of olfactory bulbs and all cortex except the pyriform lobe and a fragment of the frontal portion of the neocortex of one hemisphere.

C. Left neocortex removal supplemented by ablation of the olfactory bulbs and of the frontal half of the neocortex of the right hemisphere. This male was able to mate even after enucleation of the eyes.

D. The brain of hemidecerebrate rabbit. This animal showed normal mating behavior.

E. Bilateral neocortex removal. Olfactory bulbs ablated. This male never mated after inactivation of the olfactory system.

F. Dorsal aspect of brain of a completely decorticate female. Olfactory bulbs present but the olfactory tracts severed. On left side striatum almost completely destroyed. Superior colliculus of right side injured superficially. This animal mated and ovulated.

the right pyriform lobe. This animal was also without olfactory bulbs. The small remaining fragment of sensori-motor cortex was sufficient to enable the animal to mate. Removal of the frontal third of one cortex, even when supplemented by ablation of the neocortex of the opposite hemisphere, rendered two females and two males in no wise abnormal in respect to their sexual reactions. Here again destruction of the olfactory bulbs did not interfere with the activity. In one male and one female of this group the eyes were also enucleated. The female reacted normally but the male was usually unsuccessful in his mating attempts. He was very active and when disturbed in any way made copulatory movements. He experienced difficulty in locating the female and making proper orientations but on two occasions he intromitted successfully (fig. 1-C).

In two males and two females the pyriform lobes alone were extirpated (fig. 1-A). These rabbits mated normally after a few days.

Complete removal of the neocortex in four males and six females did not prevent normal mating. These animals still possessed intact olfactory bulbs, intact pyriform lobes and should have possessed some visual ability (van Herk and Ten Cate, 1933) and auditory sensibility if they are comparable to cats (Bard, 1934; Dusser de Barenne, 1934; Bard and Rioch, 1937). They showed marked motor deficiencies and some indication of slight diminution of tactile sensitivity due to the removal of sensori-motor areas (Brooks and Woolsey, 1938). Ablation of the olfactory bulbs of the four males abolished their mating reactions and when other rabbits of either sex were placed in their cages they either ignored them or dashed about wildly as if frightened. They never coöperated in any sexual activity although the females frequently tried to mount them. Figure 1-E shows the brain of one of these rabbits after removal of the olfactory bulbs. In one male both olfactory bulbs and the cortex of one hemisphere were removed. The animal mated after those operations but following removal of the remaining neocortex mating never occurred although the animal was kept for eighteen months. Apparently in the normal male the olfactory system is not essential to mating but after complete ablation of the neocortex olfaction is indispensable. In these anosmic males without neocortex non-olfactory sources of sensation were incapable of initiating any specific sexual activity.

The six females from which all neocortex had been ablated continued to accept males after removal of the olfactory bulbs. In one of them all possibility of visual or auditory responses was also destroyed by extirpation of the end organs, but sexual activity continued and ovulation followed mating. Such operations abolish mating behavior in the male. There are at least two possible explanations of this difference between the two sexes. First, the male's mating behavior requires a more exact orientation and involves a more complex and delicately coördinated set of activities

than is required of the female. This greater complexity of behavior would conceivably necessitate a more exact sensory discrimination and would be dependent upon higher levels of integration. Secondly, since the female is the more passive partner in mating, special senses are apparently of lesser importance to this sex than they are to the male. It seems reasonable to assume that somatic stimuli (cutaneous, deep, proprioceptive) connected with the experience of being mounted and clasped by the male constitute an effective stimulus to the female. These sources of stimuli are not abolished by the operations described above.

Three completely decorticate females (rhinencephalic cortex as well as neocortex removed) mated but only one of these ovulated as a result of coitus. These animals did not eat spontaneously and slowly lost weight until killed. They were kept for periods of from four to six weeks. One which would not mate spontaneously was given estrogenic material in the form of Progynon-B (injected intramuscularly). As a result of the injection she mated frequently but ovulation did not follow. The other two mated a few times shortly after the operation. They showed less excitement than normal females but did perform the characteristic mating responses. One of the animals (fig. 1-F) ovulated but the other failed to do so, apparently because of her poor nutritive condition. The removals were grossly identical in extent.

Three female rabbits from which the sacral cord and the lowest lumbar segment had been removed ovulated after coitus. In another it was found that transection of the cord through the lowest lumbar segment did not stop the ovulatory response to mating. Even when these procedures were supplemented by complete abdominal sympathectomy, hysterectomy and extirpation of the proximal half of the vagina, coitus was still followed by ovulation. All these preparations exhibited signs of full anesthesia and analgesia of the vagina, vulva and of the skin surrounding the vulva. This was tested by the application of strong electrical and mechanical stimuli. The animal did not respond in any way to these traumatizing stimuli. The existing paralysis of the sphincters and bladder tended to confirm the completeness of the denervation. Some intrinsic bladder and sphincter tone developed after several weeks. These experiments prove that under normal conditions specific genital stimulation is not an indispensable factor in the induction of mating behavior and ovulation, but its importance may become greater when other sources of sensation have been removed. In one of these four females removal of the olfactory bulbs and destruction of vision did not abolish mating. Other sensory clues were evidently sufficient to produce excitement. This animal on one occasion mounted another female and reached a peak of excitement which resulted in ovulation.

Females whose hind legs had been paralyzed by lumbar (4 rabbits) or

thoracic (2 rabbits) cord section failed to ovulate, though males mounted them, intromitted, ejaculated and fell off when the paralyzed animals were placed in such an attitude that this was mechanically possible. In three females both hind legs were completely denervated. For a few days the paralyzed limbs were completely flaccid but as muscular atrophy developed the legs became rigidly extended, possibly because of the greater strength of the extensor muscle group, and the joints became ankylosed. This extension of the hind legs made general movement difficult for the animals; they failed to ovulate after coitus. Two animals with almost completely denervated limbs did mate and ovulate. These animals were capable of executing some hip movement and did respond when mounted. Such observations indicate that the female must coöperate if the sexual activity and associated excitement is to be effective in inducing ovulation.

Discussion. These experiments suggest that several factors coöperate in the production of sexual excitement and the resulting activity. If the neocortex or any considerable portion of it is intact the animals are not dependent on olfactory stimuli or vision. After removal of the neocortex of male rabbits the olfactory apparatus is essential to the initiation of mating behavior. The somewhat impaired tactile sensibility and the remaining sources of stimuli are not adequate to arouse a sufficient degree of sexual excitement to induce a male with neocortex ablated and the olfactory bulbs or tracts destroyed to mount and copulate. These experiments likewise show that the female rabbit becomes sexually excited when stimulated in any one of a variety of ways. Many types of sensation probably normally play a part in the initiation of excitement but practically every one of them is dispensable provided some other source of stimuli remains. In the normal animal olfactory, visual, auditory and genital stimuli must be important but in their absence the female can be excited through other channels. Removal of the cortex creates numerous sensory and motor deficiencies but these are not sufficient to abolish excitement, mating behavior and ovulation when the animal is mounted by a male. The afferent, central and motor components of the mating response are sufficiently intact to permit a practically normal reaction.

The results obtained lead to the conclusion that ovulation occurs as a result of intense sexual or emotional excitement rather than as a result of a reflex initiated by stimulation of any specific group of sensory endings. A considerable mass of evidence can be marshalled in support of this contention. Since ovulation normally follows coitus it has been thought that it might be dependent upon genital stimulation. Marshall and Verney (1935) have produced ovulation by strong repeated stimulation of the lumbo-sacral cord but no one has been able to produce ovulation merely by mechanical stimulation of the vulva or vagina. Mating frequently fails to cause ovulation when the female does not become intensely excited.

Parks and Fee (1930) found that anesthetization of the vaginal and vulval region with "percain" did not prevent mating and ovulation. The experiments here described show that females with deafferented genital regions ovulate when mounted and when excited sexually. Sexual excitement is hard to arouse if the female cannot coöperate in the sexual activity. Animals with hind legs and hips completely paralyzed fail to show signs of sexual excitement and fail to ovulate when forced to receive males. An additional piece of evidence in support of this theory of the importance of excitement is the observation that when one female mounts another ovulation follows if after executing male-like copulatory movements she falls off as does the male on ejaculation. The afferent stimulation pattern in these cases must be quite different from that of normal mating. There is no intromission or genital stimulation; the animal is not clasped or mounted; olfactory stimuli are probably quite dissimilar. Despite this dissimilitude of stimulation intense sexual excitement unquestionably develops and ovulation ensues. Similar inferences can be drawn from the occasionally-reported examples of isolated females which become excited by proximity to males or other rabbits and ovulate without any mating behavior whatsoever. In these cases the ovulatory stimulus must be chiefly psychic or emotional.

Excitement may normally cause an orgasmal contraction of uterus, vagina or other genital tissue. This in turn may reflexly initiate ovulation in some way. However, hysterectomized animals with truncated vaginas continue to ovulate. The manner in which ovulation is induced is not fully known. Apparently in the rabbit there is some nervous mechanism involved in the excitation of the endocrine activity (increased secretion of gonadotropic substance from the anterior pituitary, Free and Parkes, 1929; Smith and White, 1931; Brooks, 1937) known to occur. It is felt that this work supports the thesis that ovulation in the rabbit is the direct result of strong sexual excitement and that this excitement can be initiated by stimuli reaching the higher subcortical centers from a variety of sensory endings. In the female the cerebral cortex is not essential to the initiation of the excitement nor to the normality of the response.

SUMMARY

Bilateral destruction of the labyrinths and auditory apparatus, enucleation of the eyes and removal of the olfactory bulbs does not abolish sexual activity in either the male or female rabbit.

Ovulation occurs normally following coitus in rabbits whose sacral cords have been removed. Even when this denervation of the genital region is supplemented by complete abdominal sympathectomy, hysterectomy, and extirpation of the proximal half of the vagina coitus accompanied by signs of emotional excitement results in ovulation.

Males which have undergone bilateral removal of all neocortex mate. After ablation of the olfactory bulbs such animals do not mate.

Females continue to mate and ovulate after removal of the neocortex and destruction of the olfactory bulbs. Three completely decorticate females exhibited typical mating behavior and one ovulated following coitus.

Evidence in behalf of the interpretation that ovulation in the rabbit is dependent upon the development of sexual excitement is discussed. It is felt that in the female this excitement can be induced by a variety of stimuli.

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INSULIN AND GASTRIC MOTILITY

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Bulatao and Carlson (1) first demonstrated that insulin was capable of stimulating gastric motility in normal fasting dogs. This report was corroborated by Quigley, Johnson and Solomon (5) who found that insulin also increased hunger contractions in normal fasting man. Quigley and his co-workers (3, 4, 5, 6, 8, 9, 10) in a series of papers have now shown that while insulin increases gastric activity in normal and splanchnicotomized animals, after vagotomy there is definite inhibition. A Heidenhain pouch which is generally believed to be without vagus innervation, was inhibited at the same time the stomach itself was being stimulated. No action was noted on a pyloric pouch which was assumed to have all nerve connections.

Since the results did not correlate with the sugar content of the blood the most likely explanation of insulin action seemed to be that it caused a stimulation of both vagal and sympathetic central nervous mechanisms, the former predominating. After vagotomy the inhibiting influence of the sympathetics was unopposed. On the other hand the action of insulin could also be explained on a peripheral basis. After vagotomy the gastric musculature is much depressed and it might be that it was the reduction in irritability of vagal endings that had prevented stimulation. The active inhibition seen after vagotomy might likewise be on some kind of terminal endings.

In short it is not yet clear whether the action of insulin on gastric motility is central or peripheral. The problem can be advanced a few steps farther by using animals which are both vagotomized and splanchnicotomized, and in which the adrenals and coeliac ganglia have been removed. The present report deals with such experiments.

METHODS. All operations were carried out with the usual aseptic techniques. The vagi were resected directly above the diaphragm in 9 dogs. The greater and lesser splanchnics were destroyed as they passed over the cruri of the diaphragm. At the same time the lumbar chains were removed from the first to the fourth ganglion. The adrenals were reached by the posterior route and the coeliac ganglia were removed through an abdominal incision. The animals recovered uneventfully and were in excellent physical condition. They were trained to lie quietly with-

out restraint during all experimental procedures. Fasting periods before the experiments varied from 6 to 48 hours. The balloon method was used for recording gastric contractions. Drugs used were Eli Lilly's iletin, surgical pituitrin, adrenalin and 20 per cent glucose.

RESULTS. One animal was studied after splanchnicotomy before vagotomy. As in the normal, insulin was found to increase gastric activity. Seven animals were studied after vagotomy before splanchnicotomy. In these insulin regularly produced inhibition. These results are in agreement with those reported by Quigley.

In 9 animals which had undergone double vagotomy and splanchnicotomy 53 insulin injections were made. Of these 9 were intravenous and 45 subcutaneous. The doses used varied from 0.8 to 15 units. The

TABLE 1

Gastric inhibition following insulin injections in vagotomized and splanchnicotomized dogs

Doses from 0.8 to 15 units

DOG NUMBER	NUMBER OF EXPERIMENTS	INHIBITION OBTAINED	LATENT PERIOD FOR CUTANEOUS INJECTIONS—AVERAGE IN MINUTES
1	8	6	21 5
2	3	2	25 0
3	10	9	19 0
4	3	2	18 7
5	8	8	20 7
6	7	6	17 4
7	2	2	18 0
8	4	4	6 0
9	8	6	12 9
Total 9.....	53	45	18 7*

* Average.

data are presented in table 1. Gastric motility was inhibited after all of the intravenous and after all but eight of the subcutaneous injections. In six of the eight negative experiments, only one unit or less of insulin had been given, evidently an insufficient amount.

Intravenous injections of insulin produced almost immediate gastric inhibition lasting from 2 to 7 minutes. For subcutaneous injections the latent period averaged 18.7 minutes, somewhat shorter than previously reported.

In a series of 16 experiments in which blood sugar determinations were made, it was noted that all doses which inhibited gastric activity also lowered the blood sugar values. In intravenous injections the drop in the glucose level followed the gastric inhibition and motility was resumed while

the glucose level was still falling. This agrees with the work of Quigley and Hallaran (7). Our denervated animals were more than normally sensitive to insulin. They showed gastric inhibition with smaller doses than normal animals did and doses of 8 to 10 units were sufficient to produce convulsions within two hours unless special efforts were made to maintain the sugar level. Insulin was most effective after 24 hours of fasting.

Thirteen experiments were carried out in which 1 cc. of 1:1000 adrenalin was injected intravenously. Inhibition with a slight lowering of tonus began immediately and lasted 2 to 3 minutes when motility was again gradually resumed.

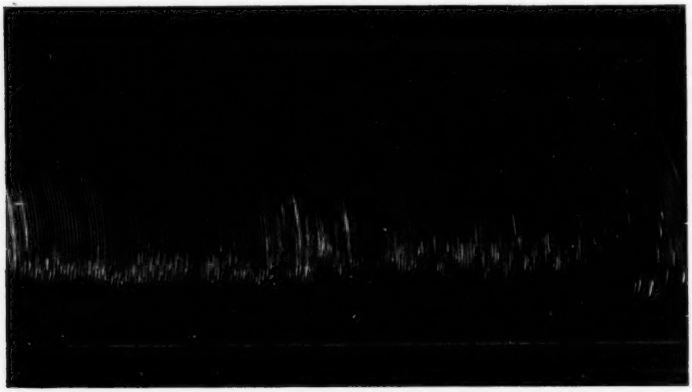

Fig. 1. Stimulation of gastric motility following the subcutaneous injection of 16 units of insulin in a dog with vagi intact but coeliac ganglia removed. Time in 5 seconds and 1 minute intervals. Injection was 3 minutes before record begins.

Surgical pituitrin was injected subcutaneously in 18 experiments. Inhibition was obtained after a latent period of 10 to 15 minutes and lasted about 5 minutes.

So far the experiments detailed agree with the idea of others that the gastric stimulation of insulin administered before vagotomy is a central effect. We may add a few observations which support this view. It has been noted in our laboratory (2) that as time goes by after vagotomy there is a considerable recovery tonus in the gastric musculature as measured by the effect of atropine in shortening emptying times. These animals, however, do not seem to regain their contractile response to insulin as they should if its disappearance depended merely on depression of the gastric musculature. Furthermore, at a time after vagotomy when the musculature has certainly returned in part to its previous condition as evidenced

by the regular hunger contractions, insulin still has not recovered its stimulating action. In some cases there is a very transient increase in rate before inhibition sets in (see fig. 2), but nothing comparable to the large increases in both rate and amplitude which are characteristic before vagotomy. (See fig. 1.)

The appearance of inhibition on injection of insulin after section of the vagi could most easily be interpreted as central stimulation of the sympathetics which had previously been masked by the vagal effect. Its persistence after subsequent section of the splanchnics, however, proves that it is a peripheral effect, although it does not prove that the action is directly on the gastric musculature. Since adrenalin shows its usual inhibitory activity following splanchnicotomy, the adrenals might possibly be stimulated by the injection of insulin. The same may be said for the pituitary. Furthermore, the postganglionic cells in the coeliac ganglion might be the point of stimulation by the insulin.

Fig. 2. Inhibition of gastric motility following the subcutaneous injection of 16 units of insulin in a dog with vagi sectioned, one adrenal removed and the other demedullated and the coeliac ganglia extirpated. Time in 5 seconds and 1 minute intervals. Injection was 6 minutes before beginning of record.

Two of these possibilities we have eliminated by direct experiment. Two dogs in which insulin gave the usual augmentation of gastric movement were vagotomized. The administration of 16 units of insulin was now shown to give inhibition. One adrenal was next removed and the other completely demedullated by cautery. On recovery insulin produced the regular inhibition. The animals were next subjected to a removal of their coeliac ganglia. As may be seen in figure 2 insulin still produced complete inhibition.

Although there is no evidence concerning the outpouring of pituitrin after insulin in sufficient quantities to be effective, the possibility of such a mechanism seems rather remote. It would seem, then, fairly well established that insulin in some way results in direct inhibition of the gastric musculature which becomes apparent on the elimination of a dominant vagal stimulation.

SUMMARY

1. As shown by others, the injection of insulin in intact dogs augments gastric motility. After vagotomy the stimulation is replaced by inhibition.

2. Even though after vagotomy the gastric musculature may recover some of its tonus and normal hunger contractions may return, the usual stimulating effect of insulin does not return. This is believed to be further evidence that the augmenting action is on the vagal center.

3. The inhibition of gastric motility on insulin injection after vagotomy persists after the splanchnics are sectioned. It is not then a central effect.

It persists after removal of one adrenal and the medullary portion of the other. It persists after removal of the coeliac ganglia. The action is therefore believed to be peripheral.

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THE ELECTRENCEPHALOGRAM OF SCHIZOPHRENICS DURING INSULIN HYPOGLYCEMIA AND RECOVERY[1]

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Since March, 1936 insulin treatments (Sakel, 1935; Müller, 1936) have been given by one of us (D.E.C.) to a group of twenty schizophrenics at the Worcester State Hospital. Patients undergoing the treatments are put to bed in an observation room and injected with empirically determined coma-producing doses of insulin. Coma usually results in from two to five hours. After roughly half an hour of coma the patient is injected with glucose, or fed Karo syrup. The treatment is repeated daily for upwards of fifty days. Patients tend to show considerable improvement in symptoms immediately following each treatment but these improvements are largely transitory. After many repeated treatments the patients often show progressively longer daily remissions until, in favorable cases, some lasting relief occurs.

We have recorded electrencephalograms by means of an amplifier and ink-writing undulator on paper tape. The electrodes were wires attached to lead pellets 2 to 3 mm. in diameter, the pellets making contact with the scalp through electrode paste (Sanborn) and held in position by collodion. The grid electrode was placed over the occiput, 2 cm. above the inion, and an "indifferent" electrode was attached to the skin behind each ear, the parallel leads from these attachments being brought together to form a common connection.

Brain wave records have been obtained at roughly thirty minute intervals during some thirty-five insulin treatments on six patients. We have also followed the progress of eleven patients from week to week during their series of insulin treatments by means of the electrencephalogram (cf. Hoagland, Cameron and Rubin, 1937).

Behavior of alpha waves during insulin treatments. In a preliminary note Hoagland, Rubin and Cameron (1936) have described the effect of insulin

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We are indebted to the Lilly Co. for generous supplies of insulin used in treating our patients. The writers are also indebted to the Biochemistry Laboratory at the Worcester State Hospital for blood sugar analyses in connection with this work.

therapy on brain waves. In this paper we shall show these effects in more detail. It has been observed by many workers (*cf.* e.g., Berger, 1933; Hoagland, 1936a; Lemere, 1936) that diseased human beings for the most part show qualitatively less regularity in brain wave patterns than do normal people. The degree of variability of brain wave records is an interesting quality of a given individual's electrencephalogram under standard conditions, i.e., any arbitrary set of conditions such, for example, as are usually employed to record alpha waves in the quiescent, awake subject with closed eyes. In many of our experiments not only was the patient instructed to keep his eyes closed, but, in addition, a folded towel was used as a blind. Following injections of doses of insulin sufficient to produce coma in three to four hours, the alpha wave frequencies are found not only to decline along a smooth curve, but the records become increasingly more irregular owing primarily to the occurrence of large, slow, arhythmic waves of fifty or more microvolts in amplitude. During coma the alpha frequency usually was reduced to 60 or 70 per cent of its pre-insulin value.

Immediately (within 10 to 30 sec.) following the intravenous injection of glucose, the irregular waves disappear and the alpha waves, almost obliterated during coma, return but at a frequency 30 to 40 per cent below the pre-insulin level. The patient rapidly recovers from the hypoglycemic condition and usually, according to our experience, his psychotic symptoms are reduced as compared to those of the pre-insulin state. He often converses intelligently and may be euphoric. The patient's condition immediately after interruption of the treatment is rather similar to that of mild alcoholic intoxication. He is usually friendly, often sentimental and flirtatious. Antagonism, refusal to talk, ideas of persecution and hallucinations are usually reduced. On the other hand, critical judgment is not good. As the affective and mildly confusional symptoms disappear, the patient often seems to be better in every respect, this effect in turn subsiding in greater or lesser degree until the next treatment. The broken-up appearance of the electrencephalogram, according to a measure to be described later during this post-sugar phase, in 30 out of 35 experiments, was decidedly less than in the pre-insulin stage. In about this same proportion of cases patients show at least transitory improvement in symptoms.

Figure 1 shows tracings of electrencephalograms during the course of an insulin medication. In an independent study published shortly after our first paper in which we described the electrencephalogram during insulinization of schizophrenics, Berger (1937) also has published oscillograms from a schizophrenic during insulinization showing slowing of alpha frequencies and the occurrence of large waves with the onset of coma.

Figure 2 shows the effect on alpha-wave frequency of insulin and sub-

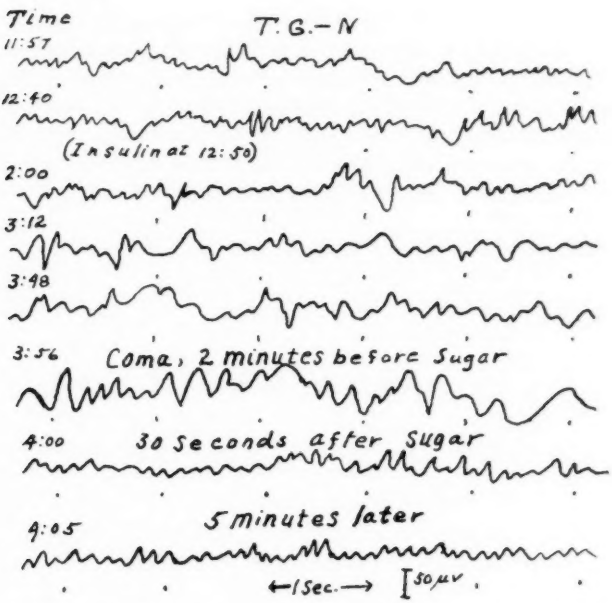

Fig. 1. Oscillograms during an insulin treatment. Note slowing of alpha frequency and increase in number of long waves as coma is approached. Note also disappearance of long waves immediately after sugar and that the base line is then more stable than before insulin.

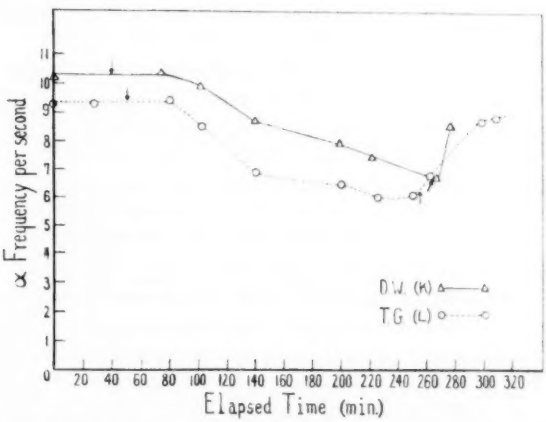

Fig. 2. Typical time course of alpha frequencies during insulin treatments. Two experiments on different patients. Insulin was injected at the arrows on the left; glucose was injected at the arrows on the right.

sequent sugar injections in two typical experiments on different individuals. Some half hour after a dose of insulin sufficient to produce coma in approximately three hours, the alpha frequencies progressively decline. The points on these and subsequent alpha-frequency curves were obtained by averaging alpha frequencies for some 50 to 80 seconds of record. These mean frequencies were then plotted against the time that the particular records were taken; the zero time was that corresponding to the first pre-insulin record.

Not only do the alpha waves decrease in frequency with insulinization, but the percentage of the time that they are present also decreases in most cases, although even in deep coma some alpha waves usually remain (*cf.* fig. 1). That they are true alpha waves may readily be verified at any time prior to deep coma by the fact that they can, in most patients, be inhibited by having the patient open his eyes in the light.

The slow random waves which increase as coma is approached are due in the main to potentials of from one-fifth to one-half of a second in duration, often causing the whole base line, including superimposed alpha waves, to fluctuate considerably. These slow waves have been found to be correlated with a number of conditions of impaired consciousness and Walter (1936) has found a preponderance of them associated with brain tumors. He has called them delta waves. In general we have found untreated schizophrenic patients to show rather more delta waves than do normals (Hoagland, Cameron and Rubin, 1937). Not only do these slow fluctuations increase markedly with the progress of insulinization but, at the conclusion of the coma as a result of sugar injection, the large fluctuations usually become less than they were before insulin. To measure this variability of the records the following method was devised.

Definition and measurement of the "delta index". A meter length of record is measured and a pencil line is drawn through the central portions of *all waves equal to or shorter than* the alpha waves within the meter sample. For all waves of distinctly longer duration than the mean alpha waves, the line is drawn over the contours of crests and troughs. The length of this pencilled line is then measured with a map-measurer and the length *in excess of 100 cm.* is arbitrarily defined as the "delta index" of the record. By this procedure the uniform alpha waves and the shorter waves, regardless of amplitude, are excluded from the measurement. This excess length appears to describe very well the appearance of disintegration of the electrencephalogram and, for this reason, we originally called it the "disintegration factor". However, to avoid confusion with psychiatric terminology we prefer to refer to this measure as the delta index, abbreviated D.I.

Figure 3 is an entirely schematic representation to illustrate the method. With practice it becomes possible merely to run the map measurer over

the record in the manner described without first drawing the pencil line, repeated readings on the same sample usually checking to within 5 per cent. The alpha waves, as we have seen, may be analyzed separately for frequency only. Their elimination in the D.I. is desirable since we have not found them to show any significant correlation with clinical symptoms. Alpha waves in either normal or pathological subjects may be dominant or subdominant or even absent entirely (Lemere, 1936; Davis and Davis, 1936).

The following protocols illustrate the D.I. in successive meters of record: A normal person, T. N. W.; 1.0, 1.0, 1.0, 0.8, 1.1, 1.1, 1.4, mean 1.1 cm. A normal M. W.; 1.0, 0.8, 1.3, 1.0, 1.1, mean 1.0 cm. A normal S. H. (identical twin sister of M. W.)

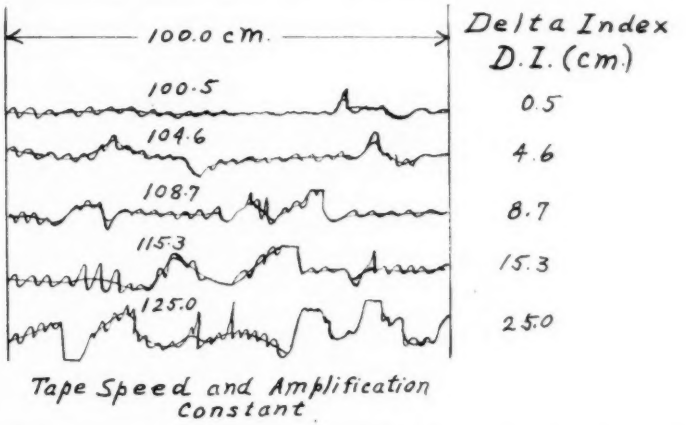

Fig. 3. Schematic representation to show method of measuring delta index. Note that *all* waves, regardless of amplitude, longer than alphas have the line run over crests and troughs. The line is passed through long waves when they become reduced in length to that characteristic of the alpha waves of the same record.

2.3, 3.0, 2.3, 2.2, 2.1, 2.0, mean 2.3. A schizophrenic patient A. S.; 15.2, 16.8, 14.0, 12.5, 17.0, 15.0, mean 15.1.

Owing to the comparative meter to meter stability of the D.I. and the fact that differences of < 1.0 cm. are not regarded as significant, we have found it expedient to measure one "typical" meter for a given determination. Repeated trials have shown that the chosen typical meter sample usually deviates by < 1 cm. from means of 5 to 10 meters of tape. Stretches of tape are discarded when observable movement of the subject occurs.

The next group of protocols illustrate typical daily hour-to-hour stability of the D.I. for eight individual schizophrenic patients not during treatment. The individual patients are represented by letters A, B, C, etc., after which, in parenthesis, is the approximate duration of the experiment. This is followed by numbers representing D.I.'s obtained at roughly equally spaced intervals throughout the hours. Between determinations, occupying about 5 minutes, the patients wore the electrodes

and waited about on the ward. A (4½ hrs.) 3.0, 4.3, 3.3, 3.8. A, on another day, (4 hrs.) 7.8, 7.5, 7.0. B (4 hrs.) 3.5, 2.0, 3.0, 4.0. C (5 hrs.) 2.7, 2.8, 2.8, 2.0, 3.0. D (6 hrs.) 4.0, 4.0, 4.0, 3.8. E (7 hrs.) 4.0, 5.7, 3.4, 3.0, 5.0, 2.2, 2.8, 3.3. F (3½ hrs.) 5.6, 7.0, 8.7, 5.7. G (6 hrs.) 7.0, 5.0, 5.0, 12.0 (probably drowsy) 6.0. H (4½ hrs.) 4.2, 3.9, 4.3.

In experiments of this kind occasionally the electrode resistance increased, as could be seen by a progressive decline in successive determinations in alpha amplitude and in D.I. over the hours. Removing and replacing the leads always corrected this difficulty. During insulin experiments we have not observed these resistance changes presumably due to the profuse perspiring of the patient during the latter half of the treatment.

Correlations of blood sugar, alpha frequency and D.I. During insulin coma the value of the D.I. may exceed 40 cm., returning after sugar and during the patient's periods of comparative lucidity often to less than

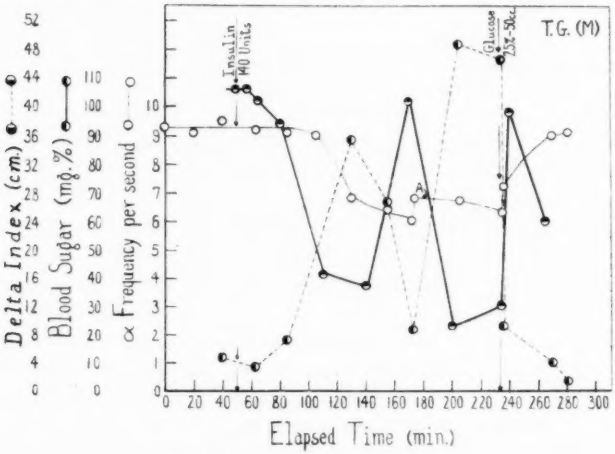

Fig. 4. For discussion see text.

1 cm., even though pre-insulin values may have been of the order of 10 or more centimeters.

Figure 4 shows the behavior of blood sugar, alpha frequency and D.I during an insulin treatment. The blood sugar level falls after the injection of insulin. In this case a rather unusual spontaneous rise in blood sugar occurs following the taking of one of the samples, to fall again to a low level during coma. When glucose is injected the blood sugar of course rises but the rise is soon followed by a secondary decline in the curve as would be expected from the known rapid utilization of sugar by the depleted tissues.

The alpha-frequency curve declines and rises with a lag of one-half to three-quarters of an hour behind corresponding changes in the blood sugar

curve. The D.I. moves almost reciprocally with the blood sugar curve. Of course at the time the record was being made the spontaneous rise in blood sugar was not known. It was learned when the analyses were returned from the biochemical laboratory the next day. However, the corresponding increase in alpha frequency was observed at the time of recording along with the sharp decrease in the disintegrated appearance of the record so that we predicted the patient was apparently arousing from his semicomatose state. Some five minutes later, at the point marked *A*, the patient became very talkative. Later the blood sugar fell to a still lower level and the patient slipped into deep coma as could be seen at the time from the rapid increase in the broken-up appearance of the record accompanying characteristic clinical coma symptoms. This experiment is unusually clear-cut in its results. Our records do not show sharp transitions between consciousness and coma. The D.I. usually increases progressively and the alpha frequency falls along a smooth curve. Coma is characterized by copious sweating, drooling, by glazed eyes and by an absence of pupillary reflexes. With practice, however, one can tell from the tape how rapidly coma is being approached and after its onset how long it has lasted.

Although the D.I. is not measured until after the experiment, it can, at the time the record is made, be estimated roughly in terms of the disintegrated appearance of the record. These qualitative observations during insulinization have proved of much value in determining the progress of the medication. Individual differences in insulin tolerance are very considerable and may vary by more than 100 per cent in the same patient in the course of a series of treatments. The electrencephalogram, therefore, is useful to the physician giving the treatment as an objective index of the patient's minute-to-minute condition.

Discussion of insulin action on brain wave behavior. From the fact that "temperature characteristics" for alpha waves in man are identical with the principal characteristic ones for cell respiration in vitro, Hoagland (1936a) has pointed out that, under *standard conditions, other things being equal,* the frequencies (f) are evidently directly proportional to the local respiration (v) of the cortical cells, i.e., $f = kv$. The present findings are consistent with this view.[2] The alpha frequency curve following as it

[2] The Arrhenius equation describes the speed of a variety of chemical processes as a function of temperature, i.e., $v = e^{-\mu/RT}$ where v is chemical velocity, e is the base of natural logarithms, T is the absolute temperature, R is the gas constant and μ is the critical thermal increment or amount of energy per mol above the average energy in the system necessary to "activate" molecules. The values of μ recorded in the literature for cell respiration in vitro for 74 groups of experiments fall in some six clearly defined modes when their distributions are plotted, some principal modes being 20 at 16,000 ± calories, 13 at 11,000 ± calories and 4 at 8000 ± calories. Some 340 diverse physiological frequencies also obey this equation, giving the same modes in

does the blood-sugar curve indicates the dependence of the frequency on carbohydrate metabolism. Kerr and Ghantus (1936) have shown decreases in both blood sugar and brain sugar in rabbits and dogs after large doses of insulin. The time lag of brain sugar behind blood sugar is roughly of the same order of magnitude as that shown in our experiments between blood sugar and alpha frequencies. It thus appears that the alpha frequencies may be regarded as a measure of available carbohydrate in the brain cells producing the rhythm.

Himwich and Fazekas (1937) have not only shown that the brain oxidizes carbohydrate, probably exclusively under normal conditions, but that hypoglycemia produced by insulin lowers both carbohydrate and oxygen utilization in brains of dogs. Blood samples from twelve dogs, taken from the longitudinal sinus and femoral artery, were analysed for glucose and oxygen. Before insulin the average value of ten observations for glucose and oxygen utilization showed 13 mgm. per cent and 8 vol. per cent respectively. During terminal hypoglycemia the respective values were 3.5 mgm. per cent and 3.5 vol. per cent. In vitro experiments with brain tissue obtained from insulinized animals have indicated a smaller O_2 consumption than do controls (Holmes, 1930). Dameshek and Myerson (1935) have shown that the arterio-venous O_2 difference of blood from human brain is diminished after the administration of insulin. Glickman (1936) found that the interval between insulin injection and convulsion in rats was greatly shortened if the animals were exposed to reduced barometric pressures. Moldavsky and Gellhorn (1937) showed that the fall in blood sugar following insulin injection produced a marked accompanying rise in blood pressure in dogs forced to breathe, for three minutes, air containing only 6.2 per cent O_2. Intravenous injections of glucose offset this pressor effect.

Rubin, Cohen and Hoagland (1937) have found good correlations between basal metabolic rates and alpha frequencies in patients injected with

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the same order as are found for cell respiration; 56 at ca. 16,000 calories, 43 at ca. 11,000 calories and 32 at ca. 8000 calories. This means that $f = kv = e^{-\mu/RT}$, where f is frequency, since $log\ f = -\mu/RT$ and the test of the equation is that log f versus $1/T$ gives a straight line of negative slope equal to μ/R.

Normals and mildly affected general paretics give, for alpha frequencies, μ = ca. 8000 calories; more advanced paretics give μ = ca. 11,000 calories, and very advanced paretics, μ = 16,000 calories. All of this "makes sense" if the modal values of μ's be regarded as energies of activation of "pacemaker" links in respiratory chains of chemical reactions in the cells. Many physiological processes have been found in which μ value shifts occur from one of the 8, 11, 16 thousand group to another of this group under changed experimental conditions. Evidently the spirochete infection shifts the chemical pacemaker from one of the reactions to another. Physiological rhythms are evidently "relaxation oscillations," i.e., oscillations in which frequency is directly proportional, *other things being equal*, to the rate at which steady state chemical events can build up critical discharge potentials (see Hoagland, 1936b).

thyroxin. This would also be consistent with the present hypothesis that alpha frequencies are proportional to the rate of cell respiration, as would the observations of Lindsley and Rubenstein (1937) of a correlation between alpha frequencies and total oxygen consumption in one thyroxinized subject.

Deprivation of O_2 by N_2 breathing in normals may, in extremes of deprivation, produce some reduction in alpha frequency and, in lesser degrees of deprivation, a very pronounced qualititive appearance of increase of numbers of delta waves (Gibbs, Davis and Lennox, 1935; Lindsley and Rubenstein, 1937). When loss of consciousness occurs from O_2 lack, the records closely resemble our coma records produced by sugar lack. The increased disintegration (i.e., number of delta waves) is, however, much more pronounced than is the decrease in alpha frequency. This last only appears in extreme cases of O_2 lack. We have confirmed these findings in patients who rebreathed air from a Douglas bag freed from CO_2.

We have used an occipital grid lead since this is known to give brain waves most free from disturbance due to eye and other facial movements and also to give the clearest alpha records. With some patients we have also recorded from a frontal grid lead immediately after occipital recording. Almost always the D.I. is larger with a grid lead on the vertex or frontal regions. This indicates that the large waves arise in the more forward parts of the head. In several of these experiments, with successive leads, we have noted carefully the absence of all extrinsic eye movements. Travis and Gottlober (1937) have reported great day-to-day uniformity in the qualitative appearance of electrencephalograms in normals. From studies of three normals and many patients we have observed a greater day-to-day, as well as hour-to-hour, variability of D.I's. in patients than in normals. This is in keeping with the greater variability encountered in many aspects of schizophrenic behavior.

Standardization of the delta index. The following material in small type describes the method of standardizing the delta index and is essential for those who may wish to use it in experimental studies. Elsewhere (1937) we have shown that the D.I. correlates very well with the day-to-day condition of individual schizophrenics.

It is, of course, important that records to be comparable be taken at constant speed and with constant amplification although a simple correction factor may be obtained from calibrations of the instrument to reduce records of different amplification to a common basis. The excess length per meter is entirely due to vertical (voltage) components in the record for waves longer than alphas, otherwise the length would be 100 cm. and the D.I. zero. Knowing the number of millimeters deflection for, say, 10 microvolts (μv.) at different attenuator settings, one can reduce D.I.'s obtained at different amplifications to some one standard amplification by multiplying by a calibration constant. This constant is the ratio of mm./μv. for the standard attenuator setting over the mm./μv. of the non-standard setting. Our apparatus gives a

linear relation of μv. to mm. over our working range and we have verified the validity of this amplification correction factor experimentally. Our standard amplification has been 1.5 mm. = 10 μv. For the most part we have run at this standard amplification, calibrating frequently.

Our tape speed has been 27 mm. per second. Since for faster tape speeds there would be proportionately fewer large waves per meter, one can readily reduce D.I.'s

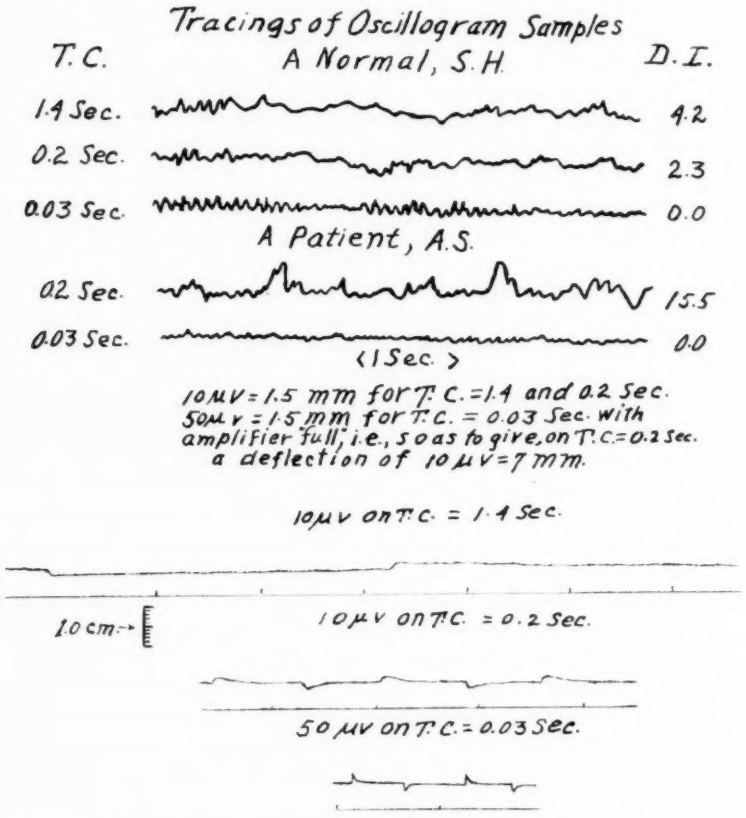

Fig. 5. Calibration data and changes in excess factors with amplifier "time constant." For discussion see text.

obtained at different tape speeds to a common basis by assuming an inverse relation between them and tape velocities. Throughout this paper we have expressed D.I.'s in centimeters and have made no attempt to express them in μv. The mathematical relation of D.I. in cm. to μv. has not been determined.

In addition to standards of amplification and tape speed, a third important standard is that of the "time constant" of the amplifier. This is fixed by the size of the smallest coupling condenser and grid leak resistance and is the length of time re-

quired for a rectangular potential impressed across the electrodes to fall along an exponential curve to half value. Our standard time constant has been 0.2 second and we have compared D.I.'s at the same amplification and tape speed at the three different time constants of 1.4 sec., 0.2 sec., and 0.03 sec.

The results of a number of experiments enable us to say that increasing the time constant of the amplifier from 0.2 sec. to 1.4 sec. roughly doubles D.I.'s of the order of 1 or 2 cm. However, for persons giving D.I.'s of 12 or 15 cm. this same change in time constant increased the D.I. some 6 or 7 fold. This disproportionately greater effect of time constant for higher values of D.I. is due to the larger number of long, irregular waves which would be less accentuated with the shorter time constant. The practical consequence of this is to indicate that for a smaller time constant than 0.2 sec. one would expect smaller magnifications of *differences* between D.I.'s of different sizes than we have obtained. For larger time constants the differences between large and small D.I.'s as we have found them with time constant = 0.2 sec., would be accentuated. All D.I.'s, regardless of magnitude, we have found to be *zero* on the time constant of 0.03 sec., despite the fact that alpha waves appear without much distortion. To show the alpha waves clearly at about the same size as with the time constant of 0.2 sec., it is necessary to run at full amplification.

Figure 5 shows oscillogram samples for different values of time constants along with actual, inked-over calibrations. For those wishing to measure D.I.'s and compare them with our results, it is important to match our conditions approximately. It should be noted that, except for time constant of 0.03 sec., our calibrating pulse, though applied rectangularly, shows some damping on the upswing. This damping is due to the impedance of our internal circuit since it is not decreased by reducing further the pressure on the tape, nor can it be further overcome to any appreciable extent by increasing the resistance in our undulator circuit. The effect of the intrinsic damping in our circuit would tend to produce rather lower pen displacements for a given impressed voltage compared to a less damped recording system. This has been demonstrated by a comparison of our calibrations with those of the apparatus used by Dr. and Mrs. Hallowell Davis. To match our amplifier characteristics it was necessary for the Davises to limit the frequency characteristics of their amplifier by introducing a suitable condenser between the input grid and ground. When this was done our calibration of 10 μv. = 1.5 mm. was superimposable on theirs at this value.

We have found that the D.I. depends also, to some degree, on electrode size. If saline moistened pads 20 x 20 mm. are used instead of the lead pellets of only a few square millimeters in area, the D.I. often is many fold larger. This is probably because the pads pick up potentials over a fairly wide area, thus producing base line disturbances.

SUMMARY

We have recorded electrencephalograms during thirty-five insulin treatments on six schizophrenic patients. The following results were obtained.

1. Electrical brain waves from schizophrenics after large doses of insulin show a progressive decline in alpha-wave frequency (Berger rhythm) of some 40 per cent, which parallels with a time lag of some minutes (circa 30) the declining blood sugar curve.

2. Sugar injected during coma restores the frequency along a smooth curve.

3. The present data, along with other evidence, is in accordance with

the view that alpha frequencies, under the standard conditions of our experiments, other things being equal, are directly proportional to the rate of carbohydrate metabolism of the cortical cells producing the rhythm.[3]

4. A new measure of waves longer than alpha waves called the "delta index" has been devised. The measure (in centimeters per cent) is a function of the excess voltage developed for these longer waves. The absolute magnitude of the delta index is a function of a number of characteristics of the recording system. These matters are considered in connection with the standardization of the index.

5. The delta index follows a roughly inverse relation to the blood sugar curve during insulin treatment.

6. Data are presented showing the degree of minute to minute and hour-to-hour stability of the delta index in a number of patients without treatments.

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[3] Since going to press Himwich et al. (1937) have shown reduced carbodydrate brain metabolism in schizophrenics during insulin hypoglycemia, thus directly confirming our present main thesis originally advanced in our 1936 paper.

AN ANALYSIS OF THE CHRONOTROPIC FUNCTION OF THE CARDIAC VAGUS NERVES

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In a previous communication (1936) we have shown that a premature stimulus applied to the pacemaker region of the atropinized turtle heart may result in a disturbance of the spontaneous rhythm so that the next spontaneous discharge of the pacemaker comes at one normal cycle interval after the application of the shock. Since such results could be obtained under favorable conditions, the conclusion was drawn that this result represents the uncomplicated pacemaker response following premature stimulation. The appearance of a longer than normal interval between shock and the next spontaneous discharge of the pacemaker is found in many records and is attributed to conduction delay and to possible effects of electric polarization. In the unatropinized heart, it is also necessary to guard against intracardlal stimulation of vagus fibers.

It is the purpose of this paper to present the results of experiments dealing with the chronotropic functions of the cardiac vagus nerves. The conclusions drawn from these experiments offer certain implications as to the nature of the vagus effects on excitability (bathmotropic effect), on conduction (dromotropic effect) and on contractility (inotropic effect). The net result has been the development of a skeleton hypothesis which is believed to have the merit of maintaining a unified concept of the various aspects of vagal cardio-inhibition.

RESULTS OF EXPERIMENTS. a. *Effect of premature stimulation of the pacemaker.* Figure 1, diagrams A to E inclusive, indicates typical results obtained by electrical recording from a turtle heart, not atropinized and beating spontaneously. The ventricle had been cut away, as had been most of the atria. Two stimulating electrodes of silver wire were placed so as to touch the sinus. One lead-off electrode was placed between the stimulating electrodes and the other was immersed in the visceral mass. Responses both from sinus and from atrium appeared on the record. The sinus response showed a prompt, sharp deflection and was measured as at the beginning of this recorded excursion. For each diagram, the times of the sinus responses are plotted along the horizontal line by the origins

of the unlettered vertical lines. The vertical line S in each case marks the time of application of the shock to the sinus.

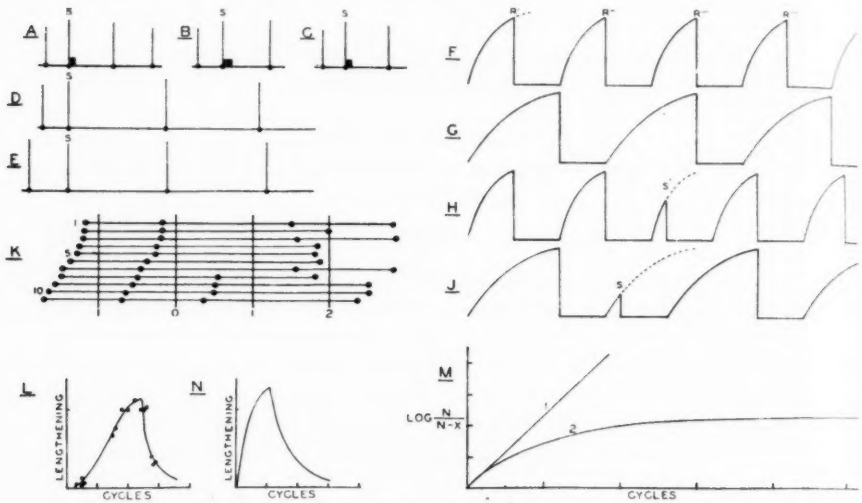

Fig. 1. A, B and C. Plotted from expt. 1/13/37. Electrical recording from turtle sinus, not atropinized. Premature stimulus applied in each case at S. Time of spontaneous discharges plotted along horizontal line. In each case, length of unlettered vertical line indicates length of preceding cycle.

D and E. From same experiment during period of cardiac slowing produced by continued stimulation of right vagus nerve in neck. Time scale same as for preceding diagrams. Ordinate scale one-half that of above.

F. Diagram indicates assumed course of recovery of normal pacemaker. Spontaneous discharge occurs at R', R'', etc. Abscissa plots time; ordinate plots percentage recovery.

G. Corresponding diagram for vagus slowed pacemaker. For diagrams F to J inclusive, the duration of systole (absolute refractory period) has been plotted as if of constant duration and equal to one-half a normal cycle length.

H and J. Diagrams for normal and vagus-inhibited pacemakers, respectively. Premature stimulation of the pacemaker at S.

K. Expt. 11/25/36, showing effect on rhythm of a single shock to right vagus in neck. For each horizontal line, shock was applied at zero time. Time of appearance of sinus response plotted by dots. Spacing of vertical lines indicates normal cycle lengths.

L. Data from same experiment plotted by method of Brown and Eccles. Ordinates plot lengthening of cycle which ends at time plotted along abscissa.

M. Plot for graphic solution of equation describing time course of effect of a single vagus volley on pacemaker recovery (i.e., equation 4).

N. Diagram of chronotropic effect of single vagus volley derived from diagram M. Latency, etc., are neglected.

Diagrams A, B and C plot results obtained without vagus stimulation. The time from the shock to the next spontaneous sinus response is longer

than a normal interval (1.47 sec.) by an amount indicated by the black rectangle at the base of the line *S*. Later in the experiment, very exact visual localization of the pacemaker region of the sinus was achieved during a period of vagal stimulation and careful placing of the electrodes on the point thus found resulted in a series of records, still without atropin, which showed no time discrepancies of the sinus intervals.

Diagrams D and E indicate results obtained while continued right vagus stimulation had slowed the heart so that the spontaneous beat interval was 2.4 times normal (i.e., was about 3.53 sec.). For D, the time from shock to next normal sinus beat is slightly longer than for the preceding or following uninterrupted cycle. For E, there is no such discrepancy. Similar results have been obtained with various degrees of vagus slowing ranging down to a rate of about four beats per minute which was achieved in one preparation.

It is found, therefore, that barring irregularities, the time from an effective premature stimulation of the pacemaker to the next spontaneous discharge is equal to one spontaneous interval whether that interval be normal or lengthened by vagus stimulation.

b. *Time course of the chronotropic effect following a single vagus volley.* Figure 1, diagram K, plots along each horizontal line, the times of beginning sinus responses, recorded electrically, immediately preceding and following the application of a single induction shock to the right vagus of a turtle. The sinus was beating spontaneously and with a normal cycle interval of 2.27 sec. For plotting, time is indicated in units of such normal cycle lengths. Zero time is taken as the moment of application of the stimulus to the vagus nerve in the neck region. The results are somewhat irregular but for the first seven series, the greatest cycle lengthening appears in the cycle in progress at the moment of application of the shock. For the last four series, it is the next following cycle which shows the greatest lengthening. Curve L plots the results of the above experiment according to the method of Brown and Eccles (1934). The time course of the inhibition so plotted yields a curve having a form somewhat similar to the single wave type of curve plotted from experiments on the cat by the above authors.

c. *Effect of frequency of vagus stimulation on rate of the heart.* Rosenblueth (1932) showed that a hyperbola is defined in several cases when effector response is plotted against number or frequency of nerve stimuli as abscissa. He regarded the finding of such a relationship as one line of evidence pointing toward the humoral transmission of nerve impulses in autonomic innervations. Among the group of effects so plotted there is included the depression of the heart rate as a result of vagus nerve stimulation. We have examined the effect of vagus stimulation rates upon the inotropic and upon the chronotropic depression of the turtle heart. Those of our records which yield good controls usually give plots lying close

along a hyperbola fitted to them (Gilson, 1933). It seems probable that an adequate theory of vagus action should be in accordance with this finding. A curve approaching hyperbolic form can be plotted for numerous asymptotically limited systems. For the present, our own material probably does not warrant a more specific interpretation than this. It should be noted that since complete inhibition must yield zero response, it is logical that chronotropic depression should be expressed in terms of heart rate rather than of cycle length. The application of the hyperbolic relationship is not precisely the same for the inotropic as it is for the chronotropic mechanism. This point will be considered later.

d. *Relationship between the two vagi and the pacemakers of the heart.* In most animals, the right vagus is much more active in producing chronotropic effects than is the left. This is in accordance with the generally recognized facts, (1) that the normal cardiac pacemaker has a right-sided locus and (2) that the effect of the uncrossed vagus nerve, particularly in the sinus region is much more intense than is the effect of the crossed nerve.

With continued moderate right vagus stimulation and a marked degree of cardiac slowing, an aberrant pacemaker often becomes effective, either assuming complete control or acting to give an irregular parasystolic rhythm. In the turtle, stimulation of the left vagus will usually stop the rhythm of this new pacemaker. In the dog the new pacemaker will often be in such a position as to give a normal form of ventricular electrocardiogram and yet be quite untouched by left vagus stimulation, or by increasing strength of right vagus stimulation. In some turtles, continued right vagus stimulation may result in a fairly regular but extremely slow rhythm.

Median sagittal section of the turtle heart according to the procedure of Garrey (1911) usually results in a pacemaker on the left side of the sinus becoming active and driving that part of the heart. Although the rate of this left-sided pacemaker is usually considerably slower than that of the normally acting pacemaker, it is occasionally found to be only a few per cent slower than the normal rate. The effect of the left vagus on such a left pacemaker is usually much the same quantitatively as the effect of the right vagus on the right pacemaker. A given pacemaker in a given heart may therefore be acted on by one vagus, by both vagi, or by neither vagus. A complete understanding of a particular preparation is had only when this fact is recognized.

Discussion. The working hypothesis outlined below cannot be regarded as offering either finality or completeness. It has been developed in an attempt to achieve a unified concept of vagal cardio-inhibition. Our interpretations of experimental results are contrary in certain essentials to the conclusions reached by Eccles and Hoff (1934) and by Brown and Eccles (1934): in other details of interpretation and in the factual details

of such experiments as offer grounds for comparison, there are no differences or differences only of a minor character.

If the course of recovery of the pacemaker following a spontaneous discharge, depends upon the simple restitution of a substance X formed from a precursor N, and if R, the rate of recovery at any time, T, depends upon the amount present of a substance P, the kinetics of the reaction may be expressed by the equation

$$\frac{dX}{dt} = kP(N - X) \tag{1}$$

or, in the integrated form

$$T = \frac{1}{R} = \frac{1}{k} \cdot \frac{1}{P} \cdot \log_e \left(\frac{N}{N - X} \right) \tag{2}$$

(In constructing the recovery diagrams of figure 1, F to J inclusive, from equation 2, zero time has been taken as at the end of the absolutely refractory period.)

If a spontaneous pacemaker discharge occurred when $\log \left(\frac{N}{N - X} \right)$ attained a certain critical value and if P were reversibly inactivated by the vagus effect, then vagal activity would result in a slowing of the heart which would depend upon the fraction of P inactivated. During a period of continued vagus stimulation the heart would show a constant slowed rate and the rate would be expected to change with stimulus frequency according to the prediction to be made from such mathematical treatment as that of Rosenblueth. The result of the state of affairs supposed to exist is indicated in diagrams F and G which plot the course of recovery for the pacemaker of a normal (F) and of a vagus slowed (G) heart. For these diagrams, the time is plotted as abscissa, values for the fraction $\frac{X}{N}$ as ordinates. Following a preceding discharge, diagram F indicates the recovery of the pacemaker to a critical level at which there occurs the next spontaneous discharge at R'. It may be that the essential recovery of the pacemaker depends on the fractional value rather than on the absolute value of X. For these diagrams 90 per cent recovery has been assumed. Simply as a convenience in plotting, we have assumed here an arbitrary, constant period of complete refractoriness (systole). The particular length of the absolutely refractory period does not affect the relationships discussed in this paper.

Premature stimulation of the pacemaker (as at time S, diagrams H and J) might result in a subnormal action potential, but the interval to the next spontaneous response would be of the same length as would that

of an uninterrupted cycle whether the cycle length were normal, as in diagram H, or for a vagus slowed pacemaker, as in diagram J.

The hypothesis was next examined for the case of the chronotropic effect following a single vagus volley. The uncomplicated recovery of the pacemaker following a previous discharge was assumed to follow a course described by equation (2). The effect of a single vagus volley was assumed to decrement in a simple exponential manner, and to produce its effect by a reversible inactivation of all or part of the substance P. As a first approximation, any period of true latency or of increasing effect due to diffusion, etc., was neglected.

Following a single vagus volley, the rate of recovery at any moment is therefore expressed by the equation:

$$\frac{dX}{dt} = k(N - X)[P_1 + P_2(1 - e^{-k_2 t})] \tag{3}$$

where X is the critical substance reconstituted during recovery
t is time (expressed in units of normal cycle length)
k is the corresponding velocity constant for the recovery reaction considered
P_1 is the part of P not inactivated by the vagus
P_2 is the part of P inactivated by the maximal vagus effect
$P_2\ (1 - e^{-k_2 t})$ thus defines the time course of recovery of active P_2 during the period of decrementing vagus effect.

The integration of equation (3) leads to the equation:

$$\log\left(\frac{N}{N - X}\right) = k(P_1 + P_2)t - \frac{kP_2}{k_2}(1 - e^{-k_2 t}) \tag{4}$$

Solution of equation 4 was obtained graphically (diagram M). A plot was made against $\log\left(\frac{N}{N - X}\right)$ as ordinate for $k\ (P_1 + P_2)t$ as abscissa (line 1), and another plot for $\frac{kP_2}{k_2}\ (1 - e^{-k_2 t})$ (line 2). The difference between the two lines at any time, t, gives the corresponding value for $\log\left(\frac{N}{N - X}\right)$ at that time. Spontaneous discharge was considered to occur when this difference value became 2.3 (i.e., $\log_e \frac{100}{100 - 90} = \log_e 10 = 2.3$). From such a graph it was possible to construct an empirical curve of inhibitory effect which would be predicted to follow a single vagus volley. Such a curve is presented as diagram N. The empirical curve N shows a general similarity of form to that of the curve plotted from actual experimental results, as L.

Under equation 4, there can be complete inactivation of P only at zero time. Actually it appears that with intense vagal effect, P may be held completely inactivated for a finite period of time, after which recovery begins to become apparent. Equation 4 should be modified to fit this fact. The experimental and the simplified empirical curves differ further with respect to the form of the early parts of the curves. For plotted actual data, the first appearance of effect is delayed, showing a latency of action. This is pointed out by Brown and Eccles and the time of latency is longer, both in their experiments and in ours, than would be expected merely on a basis of nerve conduction time. Furthermore, the experimental data plot a gradual beginning of rise of effect such as would be seen if a process similar to diffusion participated in the development of the vagus effect. Modification of equation 4 to fit these demands yields empirical curves showing excellent agreement with many of those plotted from experimental results. Many experiments fail to fit this simple system and require consideration of additional details. For example, the double wave curves of Brown and Eccles and of Fischer (1936) may indicate further diffusion effects. The appearance of these double curves recalls to mind the results obtained by Bacq and Monnier (1935) and by Eccles and Magladery (1936) on the nictitating membrane of the cat. The finding of a quick and of a slow component of response has been considered as indicative of a double mechanism, one a direct nerve effect and the other a result of humoral transmission. It is possible that for the heart there may be first a prompt vagus effect on the pacemaker and then a later effect upon the same point, mediated from a more remote locus. If the cycle relationship emphasized by Fischer and the inhibitory effect of a single vagus volley proves to be of generally constant occurrence, the phenomenon must also be explained under a complete theory.

Whether or not chronotropic inhibition and inotropic inhibition result from action upon the same chemical entities (as seems probable) it should be clearly recognized that the expression of the effect is not the same for the two cases. For the inotropic effect, there is depression of response because there is stimulation of tissue which is in a subnormal state. For the chronotropic effect, attention must be directed toward the longer than normal time required for recovery to the level at which spontaneous discharge occurs. It is probable for the pacemaker system as it is for atrial muscle that there is a decrease in the absolute recovery values attained. That the attainment of a constant percentage recovery is the factor determining the moment of pacemaker discharge appears to be consistent with the factual material now available. The concept of slowed reaction velocities in the chemical complex which determines the pacemaker rhythm not only is consistent with, but is explanatory of the results of experiments dealing with the chronotropic effects of the vagus nerves.

SUMMARY

1. As a working hypothesis, it is proposed that normally or under vagal slowing, a spontaneous discharge of the cardiac pacemaker occurs when a chemical restitution process has proceeded to a certain critical ratio level.

2. The rate at any moment of this recovery process is assumed to depend upon the concentration of a substance which is reversibly removed from acting by the action of the vagus.

3. From such a hypothesis, one would predict the results actually obtained by experiments dealing with the following:

a, the disturbance of rhythm following premature stimulation of the pacemaker, either normal or under vagus inhibition;

b, the effects upon the heart rate of varying frequencies of vagus stimulation of constant strength;

c, certain cases for the time course of the effect on heart rate following a single vagus volley. In other cases, more complicated time curves of effect appear. Such phenomena are explicable in accordance with known phenomena of diffusion. It is possible that these phenomena may operate for the cases in question.

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THE RESPIRATORY QUOTIENT AND CARBOHYDRATE METABOLISM FOLLOWING THE INGESTION OF GLUCOSE AND OF FRUCTOSE AS AFFECTED BY EXERCISE TAKEN IMMEDIATELY AND THIRTY MINUTES AFTER INGESTION[1]

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In a previous study (Haldi and Bachmann, 1937) it was found that when work was done for half an hour at the rate of 550 kilogram-meters per minute immediately after the ingestion of glucose or of fructose, the amount of carboydrate oxidized during exercise was practically the same as when water alone was taken in the amount in which the ingested sugars were dissolved. It should be of interest to know whether different results would be obtained if some time were allowed for absorption of the sugars before beginning the exercise. A comparative study has accordingly been made of the respiratory quotient and carbohydrate metabolism as affected by exercise when begun immediately and thirty minutes after ingestion of the sugars.

METHOD. The subjects of these experiments were two male adults, both well trained for this type of investigation, having served as subjects in a large number of experiments of this nature. One (W. W.) was 29 years of age, 170 cm. tall and weighed 63 kilos, the other (C. E.) of the same age, 180 cm. in height, weighed 70 kilos. On the morning of the experiments the subject came to the laboratory in the fasting state at about 7 o'clock, the last meal having been taken not later than 7 o'clock the evening before.

After a preliminary rest of twenty to thirty minutes to allow for recovery from previous exertion, the basal respiratory exchange was determined in all the experiments for three consecutive 15 minute periods with the subject in the recumbent position. Twenty grams glucose, 20 grams fructose, or a mixture of 10 grams glucose and 10 grams fructose, dissolved in 200 cc. water at 37°C. were then ingested; in the control experiments water alone was taken. In one series of experiments exercise was begun 2 minutes after ingestion; in another, 30 minutes after ingestion. The former will be referred to as "immediate exercise," the latter as

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"delayed exercise" experiments. In both series, work was done on a Prony brake bicycle ergometer for 30 minutes at the rate of 550 kilogram-meters per minute. The tension on the wheel was maintained constant and the rate of pedalling timed to the beat of a metronome. Upon completion of the exercise the subject "rolled off" the bicycle on to an adjacent couch, and without removing the mouthpiece, continued breathing into the apparatus for five consecutive 15 minute periods. In the delayed exercise experiments the respiratory exchange was deter-

TABLE 1

*Respiratory quotients during exercise begun immediately and 30 minutes after ingestion of water and sugars**

SUBSTANCE INGESTED	EXERCISE BEGUN IMMEDIATELY AFTER INGESTION					EXERCISE BEGUN 30 MINUTES AFTER INGESTION				
	Basal R.Q.	R.Q. 1st 15 minute period	Increase above basal	R.Q. 2nd 15 minute period	Increase above basal	Basal R.Q.	R.Q. 1st 15 minute period	Increase above basal	R.Q. 2nd 15 minute period	Increase above basal
Subject W. W.										
200 cc. H_2O	0.78	0.89	0.11	0.89	0.11	0.77	0.88	0.11	0.88	0.11
20 grams glucose	0.80	0.91	0.11	0.90	0.10	0.78	0.92	0.14	0.92	0.14
20 grams fructose	0.78	0.88	0.10	0.89	0.11	0.77	0.90	0.13	0.91	0.14
10 grams glucose, 10 grams fructose	0.76	0.86	0.10	0.89	0.13	0.78	0.90	0.12	0.92	0.14
Subject C. E.										
200 cc. H_2O	0.78	0.88	0.10	0.90	0.12	0.78	0.88	0.10	0.89	0.12
20 grams glucose	0.78	0.90	0.12	0.90	0.12	0.75	0.90	0.15	0.91	0.16
20 grams fructose	0.77	0.88	0.11	0.90	0.13	0.76	0.90	0.14	0.89	0.13
10 grams glucose, 10 grams fructose	0.79	0.90	0.11	0.90	0.11	0.78	0.90	0.12	0.92	0.14

* Each value is an average of three experiments.

mined also for the two 15 minute periods from the time of ingestion until the beginning of exercise, the subject remaining at rest.

In another series of experiments the respiratory exchange was followed for the same length of time with the subjects lying at rest after ingestion of water or the sugars. In these experiments gas samples were likewise collected at 15 minute intervals. The purpose of the rest experiments, as will be explained later, was to determine whether the changes in the respiratory quotient and carbohydrate metabolism induced by the ingestion of the sugars and by exercise were additive.

The volume of expired air was measured in all the experiments by the open circuit method of Carpenter and Fox (1931). Aliquot gas samples were collected in large rubber bags (*cf.* Bachmann and Haldi, 1937), and duplicate analyses of each sample made by two analysts. The analyses were repeated when they did not check within 0.02 per cent. The non-protein respiratory quotients required for calculating the amount of carbohydrate oxidized were based on the average nitrogen excretion of each subject derived from a large number of experiments.

TABLE 2

*Carbohydrate oxidized during exercise taken immediately and 30 minutes after ingestion of water and of sugars**

SUBSTANCE INGESTED	EXERCISE BEGUN IMMEDIATELY AFTER INGESTION				EXERCISE BEGUN 30 MINUTES AFTER INGESTION			
	Increase above basal			Total net increase†	Increase above basal			Total net increase†
	1st 15 minute period	2nd 15 minute period	Total		1st 15 minute period	2nd 15 minute period	Total	
Subject W. W.								
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
200 cc. H_2O	14.0	14.7	28.7		14.3	14.5	28.8	
20 grams glucose	14.9	15.2	30.1	1.4	17.6	18.5	35.8	7.0
20 grams fructose	13.0	14.3	27.3	1.4‡	15.6	17.4	33.0	4.2
10 grams glucose, 10 grams fructose	11.9	15.2	27.1	1.6‡	15.3	17.5	32.8	4.0
Subject C. E.								
200 cc. H_2O	14.3	16.9	31.2		14.4	16.3	30.7	
20 grams glucose	14.5	16.7	31.2	0	16.8	18.7	35.5	4.8
20 grams fructose	14.0	17.2	31.2	0	16.2	16.7	32.9	2.2
10 grams glucose, 10 grams fructose	15.6	16.9	32.5	1.3	16.2	18.4	34.6	3.9

* Each value is an average of three experiments.

† Obtained by subtracting the increase above the basal in the control experiments from the increase above the basal in the sugar experiments.

‡ Decrease.

RESULTS. The data presented in tables 1 and 2 were obtained from 48 experiments. To conserve space, averages only are given, each value in the tables representing an average of 3 experiments. Twenty-four other experiments, not included in the tables, were done with the subjects at rest. The data which will be referred to in interpreting the effects of exercise after the ingestion of the sugars are not described in detail, since they were substantially the same as those obtained by Carpenter and Fox (1930). The basal or post-absorptive respiratory quotients and carbo-

hydrate oxidation in both the rest and exercise experiments were obtained by averaging the results of three consecutive 15 minute periods. The basal metabolic rate from day to day was fairly uniform, the average oxygen consumption of one subject varying from 215 cc. to 230 cc. per minute and that of the other from 223 cc. to 236 cc. Normal variations of the same extent have been reported by Benedict (1934).

The respiratory quotients of exercise. In all the experiments there was a marked rise in the respiratory quotient during exercise. These quotients, as shown in table 1, ranged from 0.88 to 0.92 except in the first exercise period in the immediate exercise experiments with a mixture of the sugars on W. W., in which the quotient rose only to 0.86. In many experiments the rise in the quotient was slightly higher in the second than in the first 15 minutes of exercise.

In the control experiments with water there was but little difference in the quotients of immediate and delayed exercise. The rise of the quotients above the basal level was identical in the two sets of experiments.

When exercise was taken immediately after ingestion of the sugars the quotients were practically the same as in the control experiments with water with the exception of the glucose experiments on W. W. in which the quotients during both periods of exercise were slightly higher. The actual rise above the basal, however, was substantially the same in all the experiments. In the delayed exercise experiments, the quotients after ingestion of the sugars were higher than in the control experiments in every instance except in the second exercise period in the fructose experiments with C. E.; they were also higher than the quotients of immediate exercise in the corresponding sugar experiments. The difference in the results of the experiments with immediate and delayed exercise can best be observed by comparing the increase in the various quotients above the basal level. Although the difference in the rise above the basal in the various sugar experiments is not striking, it is somewhat greater after glucose than after fructose or a mixture of the sugars in both exercise periods with C. E. and in the first exercise period with W. W.

Since the ingestion of 20 grams of glucose or of fructose induces a rise in the respiratory quotient at rest (Carpenter and Fox, 1930), it would appear as if the larger increase in the quotients of delayed exercise after ingestion of the sugars than in the controls might have been due to a superposition of the metabolism of the sugars upon that of exercise. The greater rise after glucose than after fructose in delayed exercise, however, as contrasted with the larger increase produced by fructose in the corresponding periods of our rest experiments would indicate that such was not the case. When the net increase[2] in the respiratory exchange induced by glucose in the corresponding periods of the rest experiments

[2] The net increase was determined by subtracting the increase above the basal in the water experiments (controls) from that in the sugar experiments.

(metabolism of glucose) was added to that of delayed exercise in the control experiments (normal metabolism of exercise) the theoretical quotients thus obtained were the same as in the control experiments. This may be explained by the relatively insignificant increase in the oxygen consumption and carbon dioxide production after ingestion of the sugar as compared with the large amount of oxygen utilized and carbon dioxide evolved during exercise. As regards fructose, the theoretical quotients obtained by this procedure were only 0.01 higher above the base line than the controls, whereas those obtained experimentally during delayed exercise after ingestion of this sugar were as much as 0.04 higher. If the metabolism of the sugars were superposed upon that of exercise the theoretical quotients thus obtained should show approximately the same increase above the basal as those found experimentally in the sugar experiments during exercise; consequently, the higher quotients of delayed exercise after glucose and fructose, as compared with those of the control experiments, were not due entirely to a superposition of the metabolism of the sugars upon that of exercise. We may deduce, therefore, that the ingestion of the sugars 30 minutes before exercise led to an increase in the percentage of energy supplied by carbohydrate over and above that induced by exercise alone.

Carbohydrate oxidized during exercise. While it may be concluded from the respiratory quotients that the percentage of carbohydrate oxidized with reference to the total amount of foodstuffs burned was higher in delayed than in immediate exercise, it does not necessarily follow that the absolute amount of carbohydrate oxidized was also higher. Theoretically, if the total energy requirements of exercise had been reduced when the sugars were taken before exercise, there might have been no increase in the absolute amount of carbohydrate oxidized even though a rise in the respiratory quotient had taken place. We have, accordingly, calculated from the oxygen consumption and non-protein respiratory quotients the total amount of carbohydrate oxidized during exercise. The calculations were based on the assumption that the respiratory quotients of exercise were true combustion quotients. This assumption we believe to be justified for several reasons: 1. There was apparently no hyperventilation during exercise since there was no evidence of retention of carbon dioxide during recovery; 2, the exercise in these experiments was not sufficiently intense to produce an increase in the blood lactic acid of our subjects, as found from analyses of blood samples drawn immediately before and after exercise; 3, if lactic acid were formed from the sugars the amount of preformed carbon dioxide that would consequently appear in the expired air, would be so small relative to the total amount of this gas evolved during exercise, as to introduce no significant change in the respiratory quotient (*cf.* Bachmann and Haldi, 1937.)

The increase in the amount of carbohydrate oxidized above the basal

for the two exercise periods of the control and sugar experiments and the total net increase for the two exercise periods of the sugar experiments are shown in table 2. Since there was a slight difference in the amount of carbohydrate consumed during the various basal periods, the increase above the basal should serve as a more accurate means for comparing the results obtained in the different groups of experiments than the absolute amount of carbohydrate oxidized. In the control experiments with water the increase during exercise was the same whether exercise was taken immediately or 30 minutes after ingestion. In the immediate exercise experiments with the sugars the increase in the amount of carbohydrate burned during exercise was practically the same as in the control experiments. These results are in keeping with those previously obtained in similar experiments with 50 grams of sugar (Haldi and Bachmann, 1937). There were a few slight exceptions which, apparently, were of no significance. These exceptions occurred in the glucose experiments on W. W. in which the increase in carbohydrate oxidation was slightly greater than in the control experiments, while with C. E. it was the same; in the experiments with fructose and a mixture of the sugars on W. W. there was a small net decrease, whereas with C. E. there was no change in the fructose experiments and a slight increase with the mixture.

In the delayed exercise experiments there was a larger increase in the amount of carbohydrate oxidized after the ingestion of the sugars than when water alone was taken. With both subjects the net increase was greatest in the glucose experiments. In the experiments with fructose the increase with W. W. was the same as with the mixture of the sugars and somewhat less with C. E. The experiments on the two subjects with a mixture of 10 grams of each of the sugars, when averaged, gave an increase of 4.0 grams, approximately the same as one half the sum of the average of the increases in the experiments with 20 grams glucose and 20 grams fructose, namely, 4.6 grams. It would therefore appear as if the effects of the two sugars were additive when taken together. Carpenter and Lee (1932) have reported a similar additive effect with the subject at rest. They found that the increases in the respiratory quotient and in the amount of carbohydrate burned following the ingestion of a mixture of 20 grams each of glucose and fructose were equal to the sum of those induced by 20 grams of the sugars taken separately.

Since an increase in the respiratory quotient, as stated above, does not necessarily indicate an increase in the absolute amount of carbohydrate oxidized, further calculations were made to determine whether the increase in the amount of carbohydrate oxidation induced by the sugars was superposed upon that induced by exercise. The average net increase during delayed exercise after glucose was 5.9 grams but only 0.6 gram in

the corresponding periods of the rest experiments. In the fructose experiments the average net increase was 3.2 grams as compared with 1.9 grams at rest. These calculations therefore lead to the conclusion that the larger increase in the amount of carbohydrate oxidized during delayed exercise in the sugar experiments than in the controls, cannot be attributed to a superposition of the metabolism of the sugars upon the normal metabolism of exercise. It may be deduced that it was due for the most part to the greater availability of carbohydrate consequent upon the absorption of the sugars during the 30 minute interval between the ingestion and exercise. The larger difference between the net increase of delayed exercise and rest in the glucose than in the fructose experiments may be taken to indicate that glucose is more readily utilized than fructose during exercise.

SUMMARY AND CONCLUSIONS

A comparative study has been made of the respiratory quotient and carbohydrate metabolism during exercise begun immediately and thirty minutes after the ingestion of 20 grams glucose, 20 grams fructose, and a mixture of 10 grams each of these sugars.

The respiratory quotients of immediate exercise in the sugar experiments were not significantly different from those of the control experiments with water.

The difference between the effects of immediate and delayed exercise can best be observed by a comparison of the rise in the quotients above the basal in the various groups of experiments. During delayed exercise, the rise of the quotients in the sugar experiments was generally higher, as compared first, with that of the control experiments of this series, and secondly, with that of the corresponding sugar experiments of immediate exercise.

The amount of carbohydrate oxidized during exercise was not affected when the sugars were ingested immediately before exercise.

When exercise was begun 30 minutes after ingestion of the sugars, the amount of carbohydrate oxidized was greater than in the control experiments or in the corresponding sugar experiments with immediate exercise.

The increase in carbohydrate oxidation during delayed exercise was greater after the ingestion of glucose than after fructose.

The results obtained with a mixture of glucose and fructose in the delayed exercise experiments suggest that the effects of the two sugars were additive.

It is concluded that the increase in the respiratory quotient and carbohydrate combustion during delayed exercise was not due entirely to a superposition of the changes in the respiratory exchange and carbohydrate oxidation induced by the sugars on the normal metabolism of exercise,

but should be attributed to a large extent to the greater availability of carbohydrate consequent upon the absorption of the sugars.

The ingested glucose was more readily utilized during exercise than the fructose.

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AN EXPERIMENTAL ANALYSIS OF CENTRIPETAL VISCERAL PATHWAYS BASED UPON THE VISCERO-PANNICULAR REFLEX

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The study of sensory nervous pathways in experimental animals requires the use of objective, observable, constant reflex criteria since the animals are incapable of using the medium of language to describe their sensations. For studies of pain, previous investigators have employed a wide variety of criteria ranging from violent movements of the limbs, body and head to such phenomena as changes in skin potentials. It has been shown (Pearcy and van Liere, 1927; Ashkenaz and Spiegel, 1935) that some of these criteria are not constant.

In the present investigation, the viscero-pannicular reflex, a recently described viscero-motor reflex of the cat (Ashkenaz and Spiegel, 1935), consisting of a contraction of the panniculus carnosus muscle following adequate painful stimulation by distention of the gall bladder or duodenum, has been utilized to determine the distribution of centripetal visceral fibres of the splanchnic nerves, the particular spinal roots by which they enter the spinal cord, and the possible inter-relationships among the centripetal visceral pathways.

METHOD. In all of the experiments on the viscero-pannicular reflex, the gall bladder was stimulated by placing a rubber balloon, connected by a T-tube with a manometer and with a rubber bulb for producing pressure, into the gall bladder and sewing it in with a purse-string ligature. In the course of this work, it was found that an enormous variation in size, shape and character (lobulation) of the gall bladder of the cat exists.[1] In view of this variation, it is not surprising that it was found necessary to use a wide range of pressures in stimulating the gall bladder. The maximum pressure employed was 350 mm. mercury, while the usual mini-

[1] This is in accordance with the observations of Boyden (1922, 1925, 1926) who made a study of 2500 gall bladders in cats and found that, in some animals, the gall bladder is bi-lobed, in others it is absent altogether, in some it is very large and in others it is very small.

mum pressure required to elicit the viscero-pannicular reflex was 80 mm. mercury, although lower pressures (15 mm. mercury) were occasionally effective in eliciting a response. Boyden (1925) pointed out that the wall of the gall bladder in the cat, when normally distended with bile, becomes reduced to at least one-fifteenth of what it was when contracted; and, through forced dilatation at a pressure of 325 mm. mercury, it becomes reduced even to one-twenty-third, being then 0.1 mm. thick. The pressures mentioned by Boyden correspond quite well with those employed in the present investigation. Since the normal distention by bile may reduce the gall bladder wall to one-fifteenth its contracted size, a greater distention than this is necessary to produce pain since the normal distention is not ordinarily a painful stimulus. However, the variability in size must occasion a great variability in normal distention and these facts account for the variations in pressures found necessary to evoke the viscero-pannicular reflex.

Almost all of the animals employed in the present investigation were decerebrated by a transverse section in the midbrain, and the structures in front of the section were removed. It was believed advisable to use decerebrate preparations since the anesthesia could be discontinued and its possible effect upon the reflex avoided in such preparations, and also because the viscero-pannicular reflex, in common with other reflexes, is more easily elicited in a decerebrate preparation than in the intact animal. Because of this method of preparation, the animals had, presumably, no conscious pain. However, it is believed that the viscero-pannicular reflex is one of the most constant external manifestations of the application of an ordinarily painful stimulus to the gall bladder, and this justifies the suggestion that the centripetal pathways determined by means of this reflex may be the same as those subserving pain impulses from the gall bladder.

In the entire analysis, 60 cats were employed. In order to determine exactly the kind, number and distribution of spinal roots involved in the conduction of centripetal impulses of the viscero-pannicular reflex into the spinal cord, two types of experiment were performed: *a*, intradural section of successive dorsal roots, and *b*, transverse section of the spinal cord at various levels combined with intradural section of appropriate dorsal roots.

After decerebration of the animal and introduction of the balloon into the gall bladder, a very extensive laminectomy was performed and the spinal dura mater was exposed. Care was exercised to avoid stimulation of the spinal cord in any way during this procedure, and those portions of the cord not under immediate investigation were covered with warm physiological salt solution to prevent drying and cooling. The dura was then opened with a fine scissors in the midline of the region where roots

were to be severed. Before and after severing each root, the gall bladder was stimulated by inflation of the ballon and the response noted. The root to be cut was isolated by a fine wire and all roots were cut proximal to the ganglia. At the end of the experiment, the animal was sacrificed and the completeness of the rhizotomy verified.

EXPERIMENTAL RESULTS. a. *Intradural section of successive dorsal roots.* In this series of experiments, it was found possible to abolish the viscero-panniculer reflex by section of a sufficient number of dorsal roots on the right side in nearly all cases. In a few cases, it was necessary to cut several dorsal roots on the left side, in addition to those on the right, but *it was never found necessary to cut any but dorsal roots.*

In order to determine the upper limit of rhizotomy, it was necessary to begin with an arbitrary low root, such as lumbar 3, and continue upward, cutting successive roots until the reflex was abolished. On cutting successive dorsal roots from below upward on the right side, no change was observed in the character of the reflex until the third thoracic dorsal root had been severed. At this point the reflex became noticeably weaker, and, after the second thoracic dorsal root had been severed, it was always abolished. There were no exceptions to this upper level in any case. In order to determine the lower level, it was necessary to begin at the previously determined upper level, thoracic 2, and cut successive roots in a caudal direction. In these experiments, it was found that the reflex remained unchanged until the ninth right thoracic dorsal root was severed, when the reflex became weaker. On cutting the next or tenth thoracic dorsal root, the reflex was usually abolished. However, in some cases, it required more extensive rhizotomy to lower levels (lumbar 3, in some cases) to abolish the reflex. So, from these results, the lower level cannot be as sharply defined as the upper level. However, it is safe to say that, in no case, can a resection which does not go as low as the tenth thoracic root destroy the afferent or centripetal pathway from the gall bladder of the cat.

In order to prove that it is really destruction of the centripetal pathway and not the centrifugal pathway which abolishes the reflex after rhizotomy, a control was provided in the form of mechanical and very weak electrical stimulation of the central ends of the cut dorsal roots. It was found that stimulation of the spinal cord with an extremely light mechanical stimulus near the midline at the level of the second and also the third thoracic roots evokes a unilateral homolateral contraction of the panniculus carnosus muscle after the reflex has been abolished by dorsal rhizotomy. If the stimulus is slightly to the right of the midline, the reaction is on the right, but if the stimulus on the cord is slightly to the left of the midline at either the second or third thoracic level, the contraction of the panniculus carnosus takes place on the left. The same effects are obtained by stimulation

of the central ends of the corresponding cut dorsal roots on the right and left. At lower levels, this effect of stimulating the cord or the roots is not so marked. If the severing of the roots is begun in the middle of the thoracic region and continued in both directions from that point, the same results are obtained, with a sharp upper level at the second thoracic segment and a somewhat variable lower level at or below the tenth thoracic segment. In one experiment, it was found that severing all of the dorsal roots from thoracic 2 to thoracic 10 abolished the reflex on the right side, but the reaction still remained on the left side, even after additional right side roots were severed as far as lumbar 1. In order to abolish the left side response, it was found necessary to cut the left dorsal roots from thoracic 2 to thoracic 5. After this experiment, the usual controls of mechanical and weak electrical stimulation were employed to evoke the response on both sides.

b. Transverse section of the spinal cord at various levels, combined with intradural section of appropriate dorsal roots. This second series of experiments was performed in an attempt to determine whether there is an extra-spinal pathway for the centripetal visceral fibres in the sympathetic trunk, and also to determine the relationships among the entering pathways at various levels of the spinal cord. For example, it was believed desirable to answer the question of whether the upper inflow of impulses was as effective as the lower inflow in producing a response, and whether, if either the upper or the lower inflow were interrupted, the remaining centripetal fibres could produce a full-sized response. In order to do this, the spinal cord was transected at various levels and then the dorsal roots above the transection on the right side were cut. When the cord was transected at the level of the sixth thoracic segment, the reflex still persisted. The right dorsal roots above the transection were now severed from thoracic 5 upward. When the dorsal root at thoracic 3 was severed, the reflex became weaker, and it was completely abolished after section of the dorsal root at thoracic 2, thus establishing the same upper level as in the first series of experiments. After transection of the cord at thoracic 5, and also at thoracic 4, the reflex still persisted. In both cases, it became weaker only after section of the dorsal root at thoracic 3 and was completely abolished after section of the second right thoracic dorsal root. These results indicate that centripetal visceral impulses from the gall bladder may pass upward in pathways outside the spinal cord and enter the cord above a level of transection. It should be noted that the transection of the cord in these experiments had an effect similar to that which might be produced by transection of the sympathetic trunk at the same level, since any fibres entering the spinal cord by way of rami communicantes below the level of the transection were completely blocked from ascending in the cord to the centrifugal portion of the reflex arc at thoracic 1 and

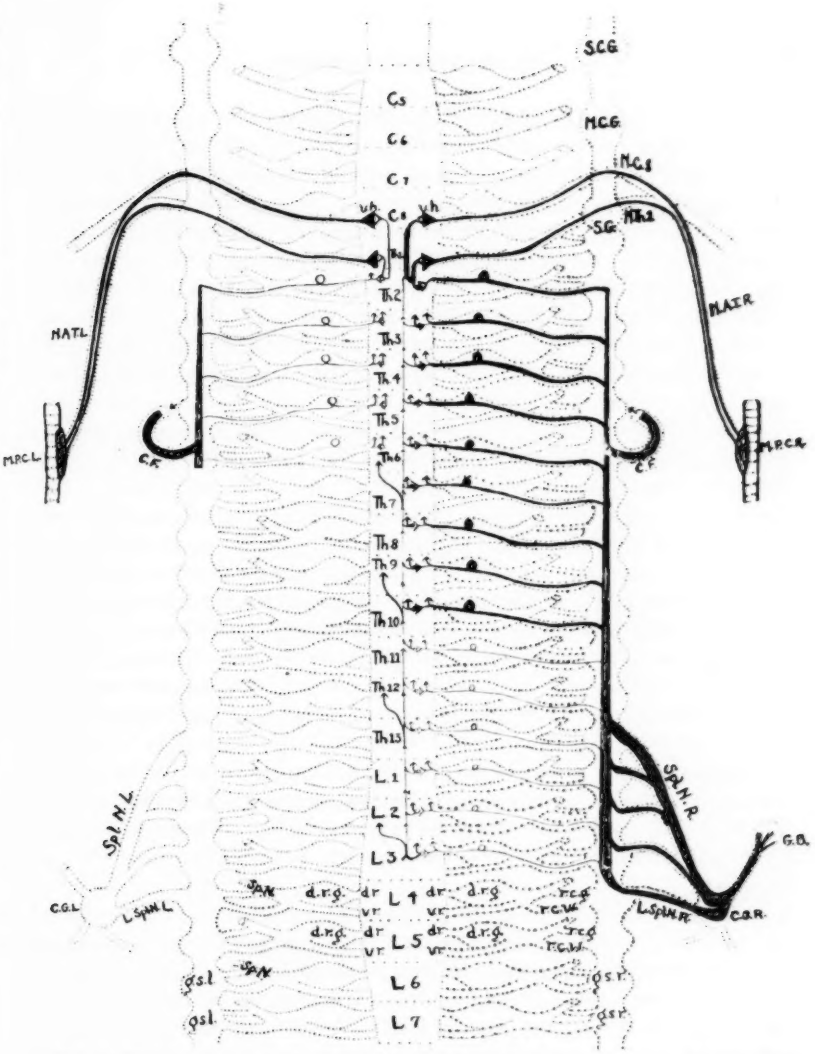

Fig. 1. The pathway of the viscero-panniculer reflex of the cat. *C. F.*, probable connecting fibres between right and left sympathetic trunks; *C. G. L.*, left celiac ganglion; *C. G. R.*, right celiac ganglion; *d. r.*, dorsal root; *d. r. g.*, dorsal root ganglion; *G. B.*, gall bladder; *g. s. l.*, sympathetic ganglia of left trunk; *g. s. r.*, sympathetic ganglia of right trunk; *L. Spl. N. L.*, left lesser splanchnic nerve; *L. Spl. N. R.*, right lesser splanchnic nerve; *M. C. G.*, middle cervical sympathetic ganglion; *M. P. C. L.*, left panniculus carnosus muscle; *M. P. C. R.*, right panniculus carnosus muscle; *N. A. T. L.*, left anterior thoracic nerve; *N. A. T. R.*, right anterior thoracic nerve; *N. C. 8*, eighth cervical spinal nerve; *N. Th. 1.*, first thoracic spinal nerve; *r. c. g.*, grey ramus communicans; *r. c. w.*, white ramus communicans; *S. C. G.*, superior cervical sympathetic ganglion; *S. G.*, stellate ganglion; *Sp. N.*, spinal nerve; *Spl. N. L.*, left greater splanchnic nerve; *Spl. N. R.*, right greater splanchnic nerve; *v. h.*, motor cell of ventral horn; *v. r.*, ventral spinal root. The darker lines in the diagram indicate the pathways found in all animals. The lighter lines indicate the pathways found in some animals.

cervical 8. In the previous series of experiments, it was demonstrated that cutting the upper spinal dorsal roots does not abolish the reflex, and this implies that impulses entering at lower levels may ascend in the spinal cord by intra-spinal centripetal pathways. Thus, it has been established that visceral sensory or centripetal impulses in the splanchnic nerve may ascend in the sympathetic trunk before entering the spinal cord or they may enter the spinal cord at lower levels and ascend within the cord.

All of the results described above have been summarized in a diagram (fig. 1). The dark lines in the diagram indicate the pathways established in most cases and the light lines indicate the variations observed in two cases.

Discussion of results. The observations of the present experiments provide a possible explanation for some of the results of attempts to relieve pain by interruption of pain pathways. The failure to relieve pain by dorsal rhizotomy (Foerster, 1927; Lehmann, 1921; Wartenberg, 1928) may be attributed to the severing of an insufficient number of dorsal roots. In no clinical case was the dorsal root resection as extensive as the results of the present investigation indicate to be necessary, although many operators realized that they were sectioning an insufficient number of roots. There was, however, no realization of the fact that resection of lower roots still permitted pain impulses to enter by upper roots and that resection of upper roots left the lower roots capable of carrying the entire burden of centripetal conduction. Failure to appreciate this fact led to misinterpretation of results and encouraged the adoption of the hypothesis that the ventral roots might subserve an auxiliary centripetal function (Foerster, 1927; Lehmann, 1921; Wartenberg, 1928). However, the present results indicate that such a hypothesis is not necessary and not tenable.

The failure to relieve pain by the operation of chordotomy or section of the ventro-lateral spino-thalamic tracts may possibly be partly explained by the existence of an extra-spinal ascending pathway in the sympathetic trunk as demonstrated in the present experiments, especially when the chordotomy is carried out at low levels. In an extensive analysis of a large number of cases of chordotomy, Foerster and Gagel (1932) suggest an extra-spinal centripetal or afferent pathway as a possible reason for the failure of some of their operations, and illustrate this possibility by a diagram (p. 53, 1932). The results of the present investigation provide experimental evidence in support of this explanation for the failure of chordotomy.

The viscero-pannicular reflex represents the type of viscero-motor reflex upon which Mackenzie (1920) based his belief that the pain symptoms of visceral disease, and especially the contraction of abdominal muscles (boarding) in disease of an abdominal viscus, could be described in terms

of visceral reflexes. It may also provide a model for the theory of Wernoe (1920, 1925) who believed that the referred somatic hyperalgesia observed in visceral disease may be dependent upon viscero-cutaneous and viscero-motor reflexes.

It is significant that the segmental distribution of the centripetal visceral pathways, as determined in this investigation, corresponds closely to the distribution of centrifugal visceral pathways as reported by Cora Senner Winkin (1922) for the vasoconstrictor splanchnic outflow from the spinal cord, by Langley and Sherrington (1891) for the pilomotor fibres in the cat, and by Langley (1903) for the pennamotor fibres of birds. In both centripetal and centrifugal fibres, the spinal segmental limits are subject to some individual variation, but both types of fibres are limited to the same (thoraco-lumbar) portion of the spinal cord. The wide segmental overlap established for the centrifugal fibres also has its counterpart in the centripetal pathways. It has been demonstrated that a single sympathetic ganglion may receive centrifugal fibres from many spinal segments, and this finds its parallel in the fact that centripetal fibres from a single viscus, such as the gall bladder, may course widely in the sympathetic trunk and enter the spinal cord in many spinal segmental levels. Thus, nearly every level of the thoracico-lumbar spinal cord receives fibres from the splanchnic nerve and sends fibres to nearly every sympathetic ganglion. The centripetal and centrifugal systems are both very diffuse.

A recent extensive comparative study of the vago-sympathetic innervation of the abdominal viscera in vertebrates by Stiemens (1934) presents further anatomical evidence in support of the viscero-pannicular pathway. The work of Bain, Irving and McSwiney (1934a, 1934b, 1935), using pupillary reactions as an index of splanchnic conduction, offers physiological evidence in confirmation of the centripetal pathway of the viscero-pannicular reflex. The papers of Ranson and Billingsley (1918a, 1918b) provide histological evidence for the wide segmental distribution of the afferent (centripetal) fibres in the splanchnic nerve. However, these workers could trace the afferent fibres, by dissection, only as high as the sixth white ramus communicans, and this is four segments lower than the highest level determined for the viscero-pannicular reflex, but the lower level which Ranson and Billingsley determined by dissection corresponds to that determined in the present investigation.

It is stated by some authors (Kuntz, 1934) that the vagus supplies the gall bladder with afferent fibres. The evidence of the present investigation indicates that these vagal afferent fibres, if they do exist, play no rôle in the viscero-pannicular reflex. Only the sympathetic afferent fibres in the splanchnic nerve are involved, since it is only necessary to cut the right splanchnic nerve to abolish the reflex.

The pathway of the viscero-pannicular reflex suggests five possible procedures for blocking centripetal impulses from the abdominal viscera:

1. Section of a sufficient number of dorsal roots, the operation of rhizotomy (Foerster's operation).

2. Section of a sufficient number of white rami communicantes.

3. Section of the sympathetic trunk in the mid-thoracic region, combined with section of a sufficient number of dorsal roots below the level of sympathetic section.

4. Section of the sympathetic trunk in the mid-thoracic region, combined with section of a sufficient number of white rami communicantes below the level of sympathetic section.

5. Section of the splanchnic nerves.

All of these procedures lead to the same result and are effective in blocking centripetal impulses but procedure 4 seems the most desirable because it involves no loss of somatic motor or somatic sensory function. Such procedures which require excision or blocking of sympathetic pathways may be relatively harmless, as demonstrated by the work of Cannon, Newton, Bright, Menkin and Moore (1929) on cats. The physiological basis and the progress in the surgery of the sympathetic nervous system have been discussed by Fulton (1930), White (1930a, 1930b, 1930c, 1935), and Gask and Ross (1934).

Adson (1936) reports excellent results with division of the splanchnic nerves (procedure 5) in one case in which three operations had been performed for biliary disease and yet no stones or active cholecystitis had been found. He believes that, if diagnostic procaine block anesthesia of the splanchnic nerves results in the sudden cessation of pain, one is justified in dividing these nerves for the relief of pain from abdominal viscera, after failure of the usual abdominal operations.

SUMMARY

1. Employing the viscero-pannicular reflex as an objective criterion of the intactness of centripetal visceral pathways, it has been demonstrated that:

A. Section of a sufficient number of dorsal spinal roots may completely block centripetal impulses from a particular viscus, such as the gall bladder.

B. Section of an insufficient number of dorsal spinal roots is ineffective in blocking centripetal visceral conduction because the remaining roots may assume the entire burden of conduction formerly carried by the severed roots.

C. Centripetal visceral fibres may follow either of two ascending pathways. They may ascend in the sympathetic trunk before entering the spinal cord, or they may enter the spinal cord at a low level and ascend within it.

D. Section of the splanchnic nerves is effective in blocking centripetal visceral conduction from the abdominal viscera.

E. There may be crossing of centripetal visceral fibres from the right sympathetic trunk to the left sympathetic trunk.

2. On the basis of the present investigation, a complete description of the peripheral centripetal pathways of a viscero-motor reflex, the viscero-pannicular reflex, has been presented.

3. These findings have been discussed in the light of the neurophysiological relations between the viscera and the central nervous system with a consideration of the problems of visceral pain, referred pain, and possible surgical procedures for the relief of intractable pain.

It is my pleasure to acknowledge my gratitude to Prof. Ernst Spiegel, in whose laboratory this work was done, for his valuable advice and unfailing coöperation, and to Prof. Frank H. Pike for his stimulating suggestions and criticism.

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THE EFFECT OF THYREOTROPIC HORMONE COMBINED WITH SMALL AMOUNTS OF IODINE UPON THE FUNCTION OF THE THYROID GLAND[1]

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The use of iodine as a palliative in the preoperative treatment of Graves' disease seems to rest at the present time upon an empirical basis. Of various hypotheses to explain the effect of iodine, two seem more worthy of attention. Very striking changes are observed in the hyperplastic thyroid of Graves' disease following the administration of relatively large doses of iodine. The depleted alveoli become distended with colloid, thus compressing the secreting cells which line the alveoli. The distended alveoli presumably compress the blood vessels in the intra-alveolar spaces. Marine (1) has suggested that the compression of blood vessels and of secreting cells produces a mechanical obstruction to the release of thyroid hormone, which may explain the remission of symptoms brought about by iodine administration. Recently Salter and Lerman (2) have proposed the hypothesis that "the therapeutic effect of iodine is indirectly a mass-law phenomenon which favors the synthesis and deposition of thyroid hormone rather than its release." The work we are reporting here would seem to lend support to the latter hypothesis.

In an attempt to assay the potency of solutions of thyreotropic hormone by measuring the effect upon the oxygen consumption of hypophysectomized rats (using the method employed by Anderson and Collip (3)), it was found that marked hyperplasia of the thyroid gland was produced after three daily injections of thyreotropic hormone without affecting the oxygen consumption of the animals, which remained at the level characterizing untreated hypophysectomized animals, i.e., approximately 35 per cent below that of normal animals. This unexpected finding led us to investigate the conditions which regulate cellular proliferation and hormone release from the thyroid gland. For this study male rats were used which had been raised in the laboratory and fed a diet consisting of whole wheat 67 per cent, fish oil 5 per cent, casein 5 per cent, alfalfa meal 10 per

[1] Aided by grants from the Carnegie Corporation of New York and the Christine Breon Fund for Medical Research. Acknowledgment is herewith made for technical assistance rendered by the Federal Works Progress Administration.

cent, and fish meal 10 per cent and salt mixture 3 per cent. The rats were hypophysectomized at two months of age and were used for this experiment three to four weeks after hypophysectomy. At this time the oxygen consumption was decreased to 34 per cent below the normal. The histological appearance of the thyroid showed marked involution. The guinea pigs used for this study were bred in the laboratory and raised on a diet of fresh vegetables and rabbit "mashettes" which consisted of ground alfalfa, ground oats, ground barley, ground corn, millrun bran, peanut meal, milk flakes, salt mixture and cod liver oil. The guinea pigs were one month of age when used, weighing between 150 and 250 grams. The oxygen consumption was measured in a Benedict multiple chamber metabolism apparatus. It was measured daily over a period of five days. Two days prior to the first reading, the animals were placed in the metabolism room which was maintained at a constant temperature of 27.5°C., where they were kept during the period of observation. After the second reading the injections were started and carried out once a day for three days. The last metabolism reading was made 24 hours after the third injection, and the animals were then autopsied. Since daily metabolism readings were made, the animals were not fasted beforehand. A single large batch of a standard alkaline solution of beef anterior pituitary extract was used as the source of thyreotropic hormone; 7 cc. of the solution represented 1 gram of fresh tissue.

The first observations in which hypophysectomized rats were injected with thyreotropic hormone are shown in table 1. A group of 66 male rats one month after hypophysectomy showed an average oxygen consumption of 131 liters per square meter body surface per 24 hours. Twelve normal male rats of the same age gave an average reading of 199 liters of oxygen per square meter per 24 hours. Thus we may consider that the hypophysectomized rats had a metabolic rate 34 per cent below that of normal rats. A group of 17 hypophysectomized rats was injected once daily for 3 days with thyreotropic hormone, the dose being 0.1 cc. per 100 grams of body weight. We had found previously that this amount of extract would produce a rise in oxygen consumption as well as hyperplasia of the thyroid in the young guinea pig. Daily readings of oxygen consumption remained consistently at low levels. On the fourth day, 24 hours after the third injection, the average reading of the oxygen consumption was 131 liters per square meter per 24 hours. There was thus no change from the preinjection reading. Another group of 24 hypophysectomized rats received four times the amount of hormone given to the first group (0.4 cc. per 100 grams body weight). Again the oxygen consumption failed to increase, the average reading of the group 24 hours after the third injection being 128 liters of oxygen per square meter per 24 hours. However, the thyroid glands of these animals showed evidence of cellular stimulation. Figure

2 is a typical thyroid taken from one of the hypophysectomized rats of group IV in table 1, which received the thyreotropic hormone. The metabolic rate remained at a level of 35 percent minus. The histological picture in this case resembles that of a normal animal and is to be contrasted with that of untreated hypophysectomized rats (fig. 1). A group of four hypophysectomized rats was injected with eight times the amount of hormone given to the first group (0.8 cc. per 100 grams body weight). The thyroid gland in these animals showed a marked hyperplasia, while the metabolic rate remained 35 per cent below normal.

TABLE 1

Observations upon the O_2 consumption of hypophysectomized rats treated with thyreotropic hormone

		NUMBER OF ANIMALS	THYREOTROPIC HORMONE	O_2 CONSUMPTION	PERCENTAGE O_2 CONSUMPTION BELOW NORMAL	THYROID HISTOLOGY
			cc. 100 gm. BW	*l/sq m. 24 hr.*		
Group I	Normal rats	12	None	199	Norm	Normal (cuboidal epithelium)
Group II	Hypophysectomized rats (uninjected)	66	None	131	−34%	Involuted (flat epithelium)
Group III	Hypophysectomized rats 24 hours after third daily injection of thyreotropic hormone	17	0.1	131*	−34%	Normal (cuboidal epithelium)
Group IV	Hypophysectomized rats 24 hours after third daily injection of thyreotropic hormone	24	0.4	128*	−35%	Cuboidal to columnar epithelium

* The oxygen consumption 24 hours after the third daily injection of thyreotropic hormone. The animals were autopsied immediately after this reading.

A search for the explanation of these findings led to an investigation of the iodine content of the rats' diet. This was found to contain 35 micrograms of iodine per 100 grams.[2] According to Remington (4), one-fifth of this amount of iodine in the diet is adequate for the maintenance of a normal functioning thyroid in the rat. The possibility then suggested itself that the abundance of iodine in the diet prevented the release of the thyroid hormone, but did not prevent the usual cellular proliferation of the thyroid produced by thyreotropic hormone. Numerous workers have

[2] We are indebted to Dr. D. Roy McCullagh, Cleveland, Ohio, for having analyzed our rat diets for iodine content.

reported upon the influence of iodine upon the stimulating effect of the thyreotropic hormone, and have shown that iodine tended to depress both the physiological and morphological changes which are produced by the thyreotropic hormone (5). In repeating their work we have found that when relatively large amounts of iodine (i.e., 3 to 15 mgm. KI) were given in conjunction with thyreotropic hormone, no rise occurred in the oxygen consumption and the thyroid gland showed alveoli distended with colloid and lined with a flattened epithelium.

The effect of small amounts of iodine upon the functional activity of the thyroid was investigated further. Since the guinea pigs raised in the

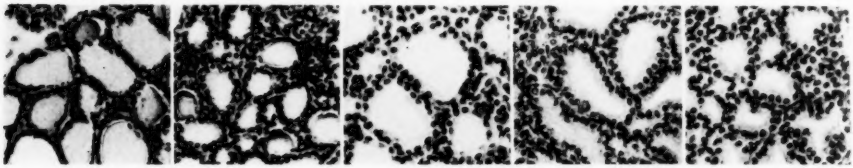

Fig. 1 Fig. 2 Fig. 3 Fig. 4 Fig. 5

Fig. 1. Thyroid of a rat one month after hypophysectomy (from group II, table 1). This shows the characteristic involutional atrophy which follows hypophysectomy. The oxygen consumption of the animal was 34 per cent below normal.

Fig. 2. Thyroid of a rat one month after hypophysectomy which was injected for 3 days with thyreotropic hormone and autopsied on the fourth day (from group IV, table 1). The thyroid gland resembles that of a normal rat. The oxygen consumption was 35 per cent below normal.

Fig. 3. Thyroid of a normal untreated guinea pig, one month of age.

Fig. 4. Thyroid of guinea pig which received three daily injections of thyreotropic hormone and was autopsied on the fourth day. There is marked hyperplasia of the thyroid. The oxygen consumption of the animal was 20 per cent above normal.

Fig. 5. Thyroid of a guinea pig which received both thyreotropic hormone and 100 gamma KI daily for three days and was autopsied on the fourth day. There was marked hyperplasia but the oxygen consumption remained unchanged from the pre-injection level.

laboratory had been found to be responsive to the thyreotropic hormone, both as to rises in the metabolic rate and to cellular proliferation of the thyroid gland, these animals were used to study the effect of combining the thyreotropic hormone with given amounts of potassium iodide. The results of this study are given in table 2. The average oxygen consumption of 87 untreated guinea pigs approximately one month of age was found to be 176 (S.D. = 10.9) liters of oxygen per square meter body surface per 24 hours. This figure was taken as the norm for guinea pigs at this age. Two control groups were used in this series. One group of eight guinea pigs was given daily doses of 100 gamma of potassium iodide

injected subcutaneously on three successive days. The daily reading of the oxygen consumption remained essentially unchanged. Twenty-four hours after the third injection the average reading of the eight animals was 163 liters oxygen per square meter per 24 hours, which was considered within the normal range. The thyroid gland showed a flattened epithelium indistinguishable from that of the resting gland of the untreated young guinea pig (normal guinea pig thyroid—fig. 3). The second control group, consisting of 25 guinea pigs, received daily doses of 0.2 cc. of thyreotropic hormone per 100 grams of body weight, which was injected sub-

TABLE 2

The effect of combined injections of KI and thyreotropic hormone upon the guinea pig

	NUMBER OF ANIMALS	AMOUNT KI DAILY	DAILY DOSE THYREOTROPIC HORMONE	O_2 CONSUMPTION	PERCENTAGE CHANGE IN O_2 CONSUMPTION	THYROID AT AUTOPSY	
						Weight	Hyperplasia
			cc. 100 gm. BW	*l/sq.m./24 hr.*		*mgm./100 gm. BW*	
Oxygen measured on normal animals.....	87	None	None	176	Norm	16	0
Control group receiving KI only.....	8	100 gamma	None	163	−6%	14	0
Control group receiving thyreotropic only.....	25	None	0 2	212*	+20%	19	++++
Group A. Thyreotropic + KI.....	13	1 gamma	0 2	207*	+17%	20	++++
Group B. Thyreotropic + KI.....	21	10 gamma	0 2	204*	+16%	20	++++
Group C. Thyreotropic + KI.....	19	100 gamma	0 2	192*	+9%	19	++++

* The oxygen consumption measured 24 hours after the third daily injection. The thyreotropic hormone and KI solutions were given simultaneously by subcutaneous route.

cutaneously on three successive days. During this time the oxygen consumption showed a steady rise; on the fourth day, 24 hours after the third injection, the average for the group was 212 (S.D. = 25) liters oxygen per square meter per 24 hours, which was a 20 per cent rise above the normal. At this time the thyroid gland showed marked hyperplasia resembling the gland of Graves' disease (fig. 4).

Against these controls three groups of guinea pigs, consisting in all of 53 animals, were studied under varying experimental conditions. All received a standard amount of thyreotropic hormone similar to that

which was given to the second control group, while the level of the potassium iodide ranged from 1 gamma to 100 gamma per day. It was found at the outset that it was essential to give the potassium iodide subcutaneously rather than intraperitoneally. By the latter route the iodide is excreted too rapidly to be effective. In experimental group A of table 2, 13 guinea pigs received one gamma of potassium iodide, in conjunction with the thyreotropic hormone. The average oxygen consumption was 207 liters per square meter per 24 hours, which was not a significant change from the control group receiving thyreotropic hormone alone. The spread in the readings was 179 to 239. Experimental group B, representing 21 guinea pigs, received 10 gamma of potassium iodide. This group showed a rise in oxygen consumption averaging 204 liters per square meter per 24 hours. Here again the readings spread from 174 to 242; however, four of the animals showed no significant rise. In experimental group C, comprising 19 guinea pigs, 100 gamma was the daily dose of potassium iodide. In this group the oxygen consumption was definitely depressed, the average being 192 liters of oxygen per square meter per 24 hours, with a spread of 151 to 217. This amounted to an average rise of 9 per cent above the normal, which is significantly less than the 20 per cent rise above normal of the control group injected with thyreotropic hormone only. The histological picture of the thyroid gland of the animals in this last group, as well as in the experimental groups A and B, presented a marked hyperplasia which was indistinguishable from that seen in guinea pigs receiving thyreotropic hormone only (fig. 3, 4, 5). The thyroid glands in all the groups of guinea pigs receiving thyreotropic hormone, showed a constant weight average regardless of the amount of iodine.

The findings presented here call attention to one of the difficulties encountered when attempting to use a physiological test such as the measurement of the oxygen consumption for assaying the thyreotropic hormone. Unless the iodine intake of the test animals is controlled, the change in metabolic rate as an index of thyreotropic activity will be quite unreliable. In fact, any method for assaying the thyreotropic hormone which measures the increase of physiological activity of the thyroid will require a uniformity of the iodine intake.

Presumably the histological changes produced in the thyroid by the action of the thyreotropic hormone are not so readily influenced by small amounts of iodine as the changes in the physiological activity of the thyroid. To what extent one must control the iodine intake when using the cellular changes in the thyroid as an index of thyreotropic activity we cannot say at the present time.

SUMMARY AND CONCLUSIONS

It is apparent that iodine plays an important rôle in the thyroid-pituitary relationship. The mechanism involved in the relationship appears

to resemble strikingly that concerned in the therapeutic use of iodine in Graves' disease. The observations recorded here show that potassium iodide administered at certain levels may prevent the release of thyroid hormone by the thyreotropic hormone without preventing the latter's effect upon the cellular proliferation of the thyroid gland.

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DIFFERENTIATION BY STRYCHNINE OF THE VISUAL FROM THE INTEGRATING MECHANISMS OF OPTIC CORTEX IN THE RABBIT[1]

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In previous papers the response of the optic cortex of the rabbit to the application of strychnine has been described (Bartley, 1933) and the form of the response to stimulation of the optic nerve has been followed by recording from various depths in the cortex (Bishop and O'Leary, 1936). We here report the effects of progressively increasing amounts of strychnine, applied to the cortical surface, upon the records of the responses to optic nerve stimulation led from different levels of the cortex.

Due to the promptness of action of the drug, to the character of the circulation of the cortex, and to the persistence of strychnine effects, a relatively steady state can be maintained for a considerable time involving a localized region, which consists roughly of a cylinder extending from the surface of the cortex to the underlying white matter. The electrode placed on the cortical surface consists of a disk of celluloid 3 mm. in diameter pierced by a fine steel needle. The surface of this disk is moistened with $\frac{1}{10}$ per cent or stronger strychnine solution, the excess blotted off, and its surface applied to the cortex after blotting the excess moisture from the cortical surface. When the desired effect has been obtained, the electrode can be replaced by a clean one after washing the surface of the brain with Tyrode solution. Since the circulation passes through vessels that extend vertically downward through the cortex, and return vertically upward, the strychnine is very promptly applied to the cylinder of tissue under the electrode, and the excess carried off with minimal distribution to the surrounding cortex, to be diluted to insignificant concentration in the veins. Since the effects are very persistent, it may be inferred that the drug is firmly fixed by the cortical tissue, and what little of it is lost by diffusion will be lost to the blood stream rather than to adjacent cortical regions. Needle electrodes thrust into the cortex 2 or 3 mm. from such a cylinder of tissue record no effects assignable to minimal

[1] This work was assisted by a grant-in-aid for research in neurophysiology from the Rockefeller Foundation.

doses of strychnine, when needles deep in the cortex within the cylinder show a definite effect. Once established, a fairly steady state persists for half an hour, and, even after small doses, returns to normal so slowly that a second application after one or two hours has a much greater effect than the first.

For purposes of this analysis, the cortex may be arbitrarily divided into three zones, based upon a convenient spacing of needle electrodes. One electrode is placed on the surface as noted above, one is thrust into the white matter below the cortex, and two more are thrust tangentially to shallower levels to afford approximately equal spacing. Between the surface and the shallowest needle below it is generally included the plexiform layer and part of the superficial pyramids. The next level includes lower superficial pyramids, the granular layer, and sometimes the upper layer of deep pyramids. The third fraction includes deep pyramids and the polymorph layer. One-half millimeter of the point of each needle is bare, the shaft insulated. Comparison records from nearby regions of the cortex into which only one needle has been thrust to the white matter indicate that the insertion of the other two needles does not materially alter the record, but the insertion of more in a small space, one above the other, may do so. The lower of two needles employed for a given record is grounded, and an upward deflection indicates positivity of the upper lead. The stimulating circuit is not grounded, or in some cases a relatively high resistance ground on a bridge circuit employed to reduce stimulus artefact does not affect the record from the localized leads significantly.

The normal records following a single stimulus to the optic nerve, from leads subtending upper, middle, and lower strata of the cortex, are diagrammed in figure 1. There can be differentiated several components (Bishop and O'Leary, l.c.): *1*, a typically diphasic spike-like figure, with which is usually fused *2*, a monophasic deflection, followed by *3*, a surface-negative component, and *4*, a surface-positive component. In the upper cortical layer, *1* appears as a nearly or quite monophasic, surface-positive spike, *2* is small or absent, *3* is absent here, and *4* may be represented by several monophasic spikes. In the middle region, *1* is about evenly diphasic, *2* is largest at this level, *3* is maximal here, and *4* is a large smooth wave, or an elevation with component spikes which do not individually return to the base line. In the lower level, *1* is mainly surface-negative, *2* has not been observed, *3* is often small and may return to the base line before the start of the subsequent *4*, which is usually smooth. Thus the 1st component of potential can be described as a diphasic one, of which the second phase is small or lacking in the upper level of the cortex, and the first phase is inconspicuous in the lower level. In some cases, the polarity of the first phase is even reversed in the lowest levels. The 2nd, 3rd, and

4th waves may be considered as a triphasic series, upon the initial wave of which the diphasic wave is superposed. The 2nd and 3rd components are fairly well confined in the middle and lower thirds of the cortex, and the 4th component is present throughout and of constant polarity. It appears to be largest for a given distance between leads in the upper middle region, that is, from the superficial pyramid and granular layers. No potential is recorded when the lower lead is above the superficial pyramid cells, that is, the fibers of the plexiform layer are not recorded at the amplification employed, either because not enough of them are active, or because the majority of them run horizontally between vertically spaced electrodes.

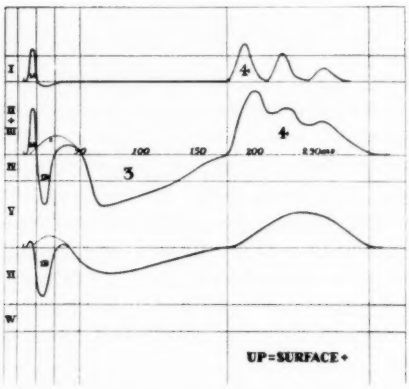

Fig. 1. Schematic diagram of potentials recorded from different strata of the optic cortex. See text.

The pathway of the impulse through the cortex. We are not in a position to assign given potential waves to the activity of specific cell types in the cortex, but we anticipate that this type of correlation will eventually be feasible. At present we are moved to suggest the distribution and certain of the properties which such cell groups might possess, as groups, in order that they should give rise to the potentials observed.

It is first necessary to arrive at some conclusion as to how an active unit may affect electrodes in its vicinity. Considering that all electrodes here employed are placed vertically one above the other in the cortex, our records are those of *vertical components* of the potential fields set up about active elements. We must here distinguish between the vertical component of a field, and the field about a vertically oriented active element. A vertically directed axon, for instance, if it passes two electrodes, will be recorded diphasically, and its two phases, in the summated response

from many such elements out of exact phase with each other, will tend to annul each other in the record. The residual differences of potential have been discussed elsewhere (Bishop, 1936). If such an element passes one electrode and terminates (or arises) between electrodes, it will render the one negative to the other to a maximum degree. A vertically directed axon (or dendrite if it produces a potential) will therefore affect the record with a vertical component of potential especially if it terminates within the inter-electrode space, or changes from a vertical to a horizontal direction there, whatever the direction of propagation along the axon. It will render negative that electrode which is nearest the active element.

A horizontally directed axon may also give rise to a vertical component of potential, and depending on its location with respect to the electrodes and the distribution of similar axons, this component will be of significant magnitude. Again the electrode nearest the active element will be the more negative. The same arguments will apply to active cell bodies, if by reason of a gradient of potential from one end to the other they should be effective sources of potential. It is thus not critical what assumptions are made for the present purpose as to the part of the neurone responsible for cortical potentials; in general, the more negative electrode will be that one in the vicinity of the greater number of active elements; and few elements will be so situated as to have no effect on any electrode.

In this sense we may speak of the distribution of intensity of the mass impulse with time during cortical response, and our records are reflections of the vertical components of the potentials set up, and not primarily of the polarity of the potential sources, nor of the direction of propagation of the impulses.

The following propositions should then be applicable. 1. If at a given instant one electrode is more negative than another, greater numbers of elements are active in its vicinity. 2. If two electrodes are isopotential, either activity is absent in the vicinity of each, or activity is present to the same extent in both regions, and by reference to other combinations of leads, one can determine which is true. 3. A *constant* difference of potential per unit distance between leads then indicates a progressively *increasing* amount of activity in the direction of the more negative leads. By means of these three principles the distribution of activity throughout the cortex can be estimated from moment to moment, and the pathway of the mass impulse indicated, by inspection of the potential records from various levels. A first approximation to such a description is presented in figure 2.

The first monophasic potential of the sequence of three represents negativity in the middle strata of the cortex, its most intense region coinciding fairly well with the distribution of afferent endings of the optic radiation. Its restriction to this region suggests cells with short processes

as its source. The surface-negative wave following this indicates a shift of the negative region upwards in the cortex. The fact that the middle strata are negative to the lower, but virtually isopotential with the upper, would indicate that the upper half of the cortex was equally negative to the relatively inactive lower half. The final wave, surface-positive from electrodes at any depth, indicates an increasing negativity from surface to white matter, which may be correlated with the increase in number of pyramid cell or other axons passing the lower of two electrodes on their way out of the cortex.

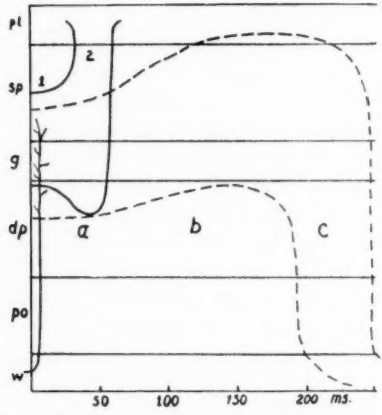

Fig. 2. Representation of the pathway of the mass impulse through the optic cortex. Diphasic, presumed visual component of the impulse, in full lines, triphasic facilitating or coördinating component, dash lines. It should be recognized that since many cells of the cortex have axons, branches of which pass both upwards and downwards, this schema describes only the propagation of loci of relative negativity, and represents only the predominant shift of the centers of activity rather than the direction of specific fiber pathways.

1, *2*, the two phases of the diphasic process. *a*, *b*, *c*, the three phases of the triphasic process.

The first phase of the diphasic potential indicates a negativity again in the middle regions near the endings of afferent fibers, which appears to extend somewhat further toward the surface than does the component considered above. The second phase shows roughly isopotentiality in the upper third of the cortex, including the surface white matter, which is all negative to the middle third, with a gradient of negativity through the lower strata. This would be consistent with a distribution of cells in the lower two-thirds of the cortex with processes extending through the upper third, to leave that immediate region of the cortex by way of the plexiform layer.

This distribution may account for the conduction of spontaneous responses under the influence of strychnine along the cortex away from the strychninized area, which are not only assignable to the elements which give the normal response to stimulation, but appear to involve the surface layers of the cortex (Bartley, 1933). It may be significant in this connection that such conduction into normal cortex requires either a high concentration of strychnine applied to a small area, or a lower concentration applied to a larger area, which should mean that it is a function of the

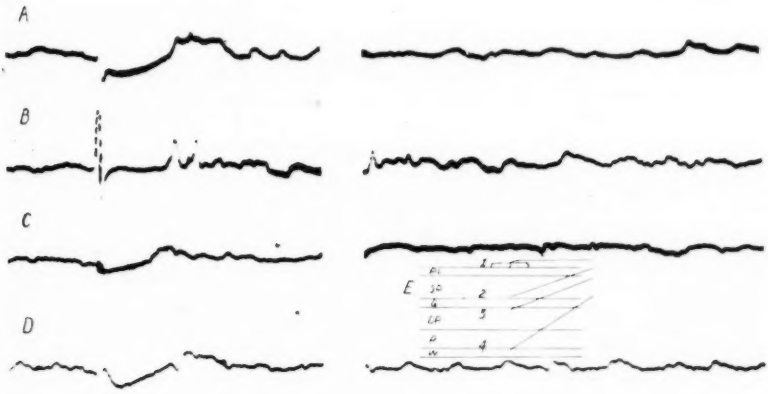

Fig. 3. Initial effects of strychnine applied locally to the surface of the cortex. *A*, from leads 1 and 4 of inset figure, *i.e.*, across the whole cortex. *B*, leads 1 and 2. *C*, leads 2 and 3. *D*, leads 3 and 4. To the right are spontaneous records corresponding. The effect on the first diphasic component, showing in *A*, appears only in the superficial leads, *B*. Records taken immediately after application of strychnine. Later all leads are affected. Inset, diagram of cortex to scale showing positions of all electrodes as checked by 7 mu histological sections. Plexiform, superficial pyramid, granular, deep pyramid, polymorph and white strata. All stimuli in this and following figures are condenser shocks or brief galvanic currents from an ungrounded circuit, the only ground being the lower of two electrodes in the cortex. Amplification of original 25 mm./mv. in *A*, and twice this in *B* to *D*. Reduced to $\frac{1}{4}$ size.

amount of cortical tissue involved, since the stronger concentration has an effect deeper in the cortex. Such conduction takes place only with concentrations of strychnine which abolish the late components of the response to stimulation, and which produce, even in the normal cortex well beyond the strychninized area, only diphasic or triphasic potentials resembling the early part of the response to stimulation.

Effect of strychnine on the cortical record. The minimal effect of strychnine (fig. 3) is to increase the amplitude of the 1st diphasic component,

with no effect on the following, nor on the spontaneous rhythmic activity that may be present. This effect is obvious after an interval of a few seconds, or about as soon as the apparatus quiets down after placing of the electrode on the surface. The upper leads are affected first, but all depths are involved promptly. The general form of the 1st component is

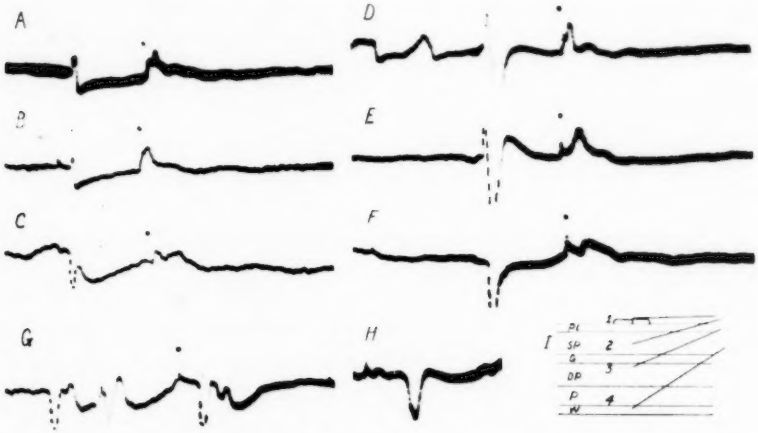

Fig. 4. *A*, *B*, *C*, before, and *D*, *E*, *F*, after strychnine, optic cortex, from positions as indicated in inset diagram. Dots over records locate shock-like deflections marking time of 240 ms. after the shock. Note that in *C* the first phase of the normal record is reversed as compared to *A*, and both phases of *A* are represented by a single downward, surface-negative deflection in *C*. The middle of the cortex is negative to regions above and below it. Increase in the amplitude of these components by strychnine does not alter this situation, but the increase is greater in each surface-negative phase than in its preceding positive phase. The later components are not affected at this stage of strychnine action. *G*, a surface-negative spontaneous wave from leads 3 and 4, followed by a stimulated wave, with a third wave occurring at the crest of the late component of the response to stimulation, at the time when facilitation to a second stimulus would have been at a maximum. The response following so closely the spontaneous wave is unique; the second fast wave in the response is not uncommon. *H*, a delayed "first" response to a stimulus, leads 3 and 4, preceded by a trace of the first surface-positive wave of the triphasic series. Each record, *A* to *F*, 1 second duration. The shocks preceding the stimulus, *B* and *F*, are due to depressing of hand key before the interruptor key closes in series. Amplification all records 25 mm./mv. Reduced to $\frac{1}{4}$ size.

not altered initially except in amplitude, and its amplitude varies with the strength of stimulus as in the normal preparation. The effect of a given dose depends upon the depth of (ether) anesthesia, and apparently different rabbits differ in sensitivity aside from this.

The effect of somewhat more strychnine (fig. 4), of a second application,

or of a more effective first application, is to further increase the amplitude of the diphasic component, the second or surface-negative phase being often increased more than the first. We have never observed an increase in amplitude of the later components more than the normal variation in amplitude would cover. The earlier waves may be increased by several hundred per cent in all levels of the cortex before any other effect occurs. The next stage is a rise in threshold, a still further increase in amplitude of the 1st diphasic wave, a decrease in the amplitude of the 3rd and 4th components without marked change in form, a later occurrence of these measured from the time of stimulation, and a reduction in amplitude of the spontaneous 5 per second rhythm. These events occur together with respect to time or to concentration of strychnine.

With still more severe involvement (fig. 5), the 3rd and 4th components disappear, the 1st diphasic component may increase to 10 times its original amplitude without marked change in form, the normal record of spontaneous activity disappears, and the response to stimulation becomes all-or-none, the threshold now being near the maximal stimulus for the normal. Spontaneous responses to strychnine, of different form from the normal spontaneous 5 per second wave, may occur before the late responses to stimulation are completely depressed. Spontaneous trains ("spasms") appear usually only after the failure both of spontaneous rhythm and of the late components of the stimulated response. Just before such spasms appear spontaneously, a single stimulus may call forth a double or triple wave, or a whole train. After such a spasm the cortex may be completely inexcitable via the optic nerve for many seconds.

Single spontaneous responses duplicate most exactly the initial components of the response to stimulation, both in form and amplitude and in differences of form as recorded from different depths in the cortex. When shortly preceded by a single spontaneous response, a stimulus of any strength is often totally ineffective. The conviction is forced upon one that the 1st diphasic component of the response to stimulation under strychnine is of the same character as that in the normal preparations, differing chiefly in amount; and that the single spontaneous responses induced by strychnine occupy the same elements of the cortex as these, in the same manner. The trains of strychnine responses are obviously composed of successions of the single ones.

When still larger amounts of strychnine are applied (a droplet of 1 per cent), after repeated trains of spontaneous waves, all sign of activity ceases and none can be induced by stimulation. Adjacent areas of the cortex may be normal, or may show spontaneous activity, presumably conducted from the margin of the now inactive strychninized area (Bartley, l.c.). Outside the range of such conduction, but still within the striate area, the strychninization can be repeated with similar results, apparently

unaffected by the first application elsewhere. With moderate doses, the effect on the response can be graded reversibly by altering the depth of anesthesia, as if the actions of strychnine and of ether were simply reciprocal, but in annulling the excitatory or depressant effects of larger doses,

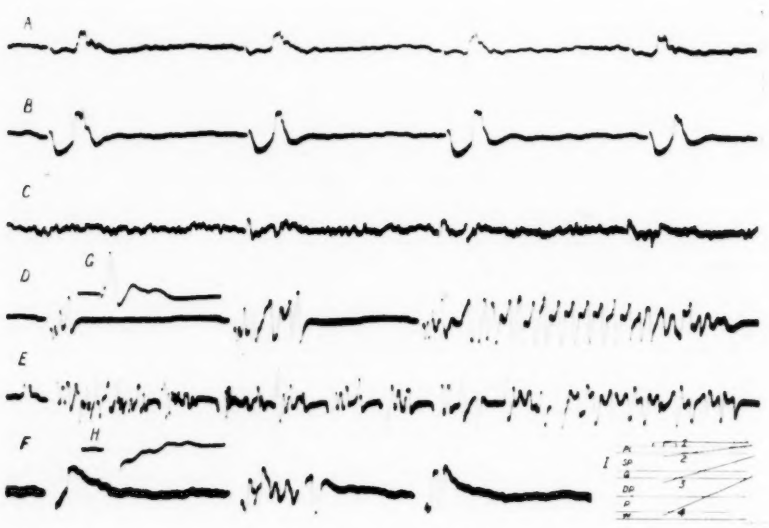

Fig. 5. Severe response to strychnine. *A*, *B*, *C*, normal responses to stimuli at 1 second intervals. Ether has nearly depressed first diphasic potential. Recorded from leads as indicated in inset. *D*, *E*, *F*, corresponding responses after severe dose of strychnine. Part of difference between records from different depths due to increasing effect of strychnine as records were taken serially a few seconds apart. Late components of response suppressed before records *D*–*F*, which were taken at ½ the amplification of *A*–*C*. *D* and *E* illustrate the cumulative effect of successive shocks at 1 second intervals. In *F*, the cortex is about to become refractory to stimulation.

G is a single response recorded as in *D* but at X3 on the time scale, taken after washing the cortex and partial recovery. *H*, same relation to record *F*. The records have no late components, which are still suppressed. In the upper cortical leads the diphasic component has a high positive phase, in the lower leads, the time of both phases is occupied by a single negative phase, as in the normal case of figure 3. In record *C* before strychnine almost no response at all appeared from the lower leads. Amplification *A* to *C*, 50 mm./mv., of *D* to *H*, 10 mm./mv. Reduced to ½ size.

generally depressant effects of anesthesia supervene, and responses to optic nerve stimulation may not be obtained.

If the initial dose of strychnine is fairly effective, the result is observed at all depths of the cortex almost immediately. With threshold doses,

and in occasional animals even with doses that are severe enough to cause spontaneous activity, the upper pairs of leads show an effect before the lower (fig. 3). For instance, the first component may be increased in amplitude up to 4 or 5 times at the upper leads at a stage when the middle leads record a wave of twice normal and the lower leads show no effect. The late 4th component may then be absent in the upper leads, low in the middle, and normal in the lower leads, and the spontaneous 5 per second rhythm parallels this. The high frequency spontaneous rhythm persists. Later all levels become involved, sometimes maintaining a gradient of effect, or a threshold dose may show a persistent gradient, to be abolished by a second application. This has been observed when, instead of the area only under the electrode, an area surrounding it ½ cm. in diameter was treated with very dilute strychnine. The gradient of effect is therefore not due to the lower electrodes lying outside the cylinder under the surface electrode. This seemed important to ascertain because of the significance such a condition might have in interpreting the pathway of the impulse through the cortex. If the upper electrodes show a blocking of a given component while lower levels do not, this component in the lower levels must not have been activated via the upper strata.

In one experiment an area ½ cm. in diameter was treated with 1/500 strychnine, which at first caused a few single spontaneous responses, and a lowering of the 4th component to ½ its normal amplitude. During recovery, a differentiation between the diphasic wave and the monophasic wave fused with it could be made. Stimuli below maximal but of constant strength then produced, apparently at random, different effects (fig. 6). Either the diphasic wave failed to appear entirely, leaving the monophasic wave uncovered (6A), or the diphasic wave was large, *i.e.*, maximal for that degree of intoxication, or a small diphasic wave was superposed on a monophasic wave (6B and C). In either type of response, the later components were identical; that is, the diphasic wave was not the necessary precursor to them. With stronger stimuli, each response contained a diphasic first wave. The normal record previous to application of strychnine showed a monophasic first wave in the upper stratum, and a diphasic wave in the middle stratum. At the transitory stage when occasional spontaneous diphasic waves appeared, a strong stimulus closely following a spontaneous wave resulted in the normal appearance of the triphasic sequence 2, 3, and 4, without the diphasic 1. In similar experiments, a first weak stimulus after a rest at this stage produced a monophasic wave, with a typical diphasic wave superposed upon the late positive component instead of in its usual order, and this dropped out after one or two stimuli at 1 per second. Further slight applications of strychnine after partial recovery reproduced most of these phenomena.

Figure 4G shows an unusual case of a spontaneous wave induced by

strychnine followed immediately by a stimulus which produced a similar wave, with a third such wave falling in the crest of the late positive phase of the response to stimulation. The deflection is monophasic downward because led from the lowest stratum of the cortex. The occurrence of such a response at the time of the positive response to stimulation has been observed repeatedly, and may be correlated with the facilitation to a second stimulus that accompanies this wave normally.

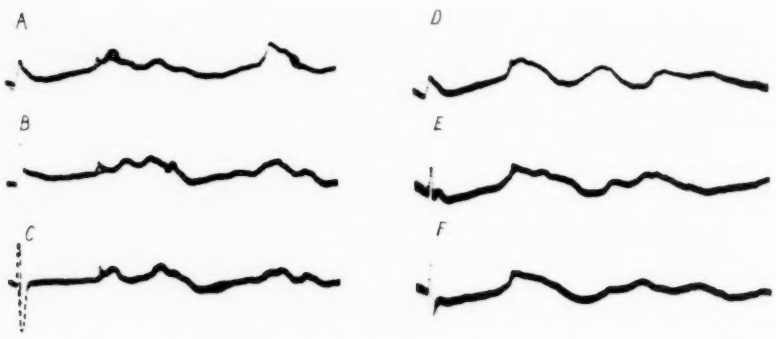

Fig. 6. Records *A*, *B*, *C*, from across the whole cortex, *D*, *E*, *F*, from electrodes one on surface and one in the granular layer, after application of $\frac{1}{5}$ per cent strychnine on an area of the optic cortex $\frac{1}{2}$ cm. in diameter. The responses at 1 per second showed a random variation in amplitude of the first diphasic wave, and the records selected are arranged in the order which shows a progressive covering up of the first surface-positive wave of the triphasic series, by this variable diphasic element. In *A* and *D* only a monophasic wave is apparent. In *B* and *E* the first wave appears to be nearly monophasic, but is really a diphasic wave superposed on a monophasic one of longer duration, the two being of such amplitudes that the first phase of the diphasic potential sums with the first half of the monophasic wave to form a spike, while the second phase and the last half of the monophasic wave annul each other. *C* and *F* show the predominance of the diphasic wave, to the extent of complete obliteration of the monophasic. Note that the later components following the monophasic wave are not affected. Deeper layers showed no strychnine effect even on the first potentials. Weaker shocks produced regular alternation in high and low diphasic waves, with constant amplitude of late components, in the upper leads. Time marks appear as shocks 180 ms. after stimuli. Amplification *A* to *C*, 25 mm./mv., *D* to *F*, 50 mm./mv. Records reduced to $\frac{1}{4}$ size.

The course of the above experiments during recovery from strychnine indicates that in the normal the first positive phase of this diphasic element must be somewhat increased by summation with the monophasic one, but that its second negative phase must be decreased, since the monophasic wave has the longer latency (fig. 6). The resultant of a diphasic plus a monophasic response could appear as a single first wave, or as one with

any degree of diphasicity. We have frequently observed in normal preparations that a first maximal stimulus produced a response with a diphasic first component, subsequent responses following stimuli 1 second apart being nearly monophasic. These results suggest that in the normal cortex the monophasic response may be present in the upper strata even though it does not appear in the record.

The organization of the cortical response. We believe that several inferences can be drawn from the action of strychnine on the cortex, concerning the normal organization of the response to a stimulus. The first is in confirmation of a previous notion (Bishop, 1936) that there must be two systems of elements in the optic cortex, one responsible for the slow spontaneous rhythm which does not produce a "visual" effect in itself, the other that involved in the "visual" or functional response to optic nerve stimulation. From the many correlations between spontaneous rhythmicity and the 4th component of the response to a stimulus, reported in previous papers, this component would seem to be closely related to the spontaneous rhythm; for instance, it may itself be repeated at the characteristic frequency after a single stimulus, and during its occurrence, facilitation occurs to a second impulse via the optic nerve, as can often be shown for the spontaneous waves. Here we find one set of potentials wherever it is represented in the cortex decidedly intensified by strychnine, at a time and concentration such that another set is everywhere and completely suppressed. Further, when the late potentials are suppressed, the spontaneous rhythm associated with them also disappears. Still further, when these responses fail, the facilitation to a second impulse which normally accompanies them is also lacking.

It might have been expected that any agent which caused so marked an increase of effect would also shorten the intervals at which the cortex could be activated, but this is not the case. If the rotator making one revolution per second is so arranged that a second stimulus can be delivered to the optic nerve at a variable time after the first, and both stimuli are made supermaximal, the second can be moved progressively earlier or later in the cycle of response to the first, and its effectiveness observed during the different phases of the first response. In the normal preparation, the second stimulus produces little or no cortical response if it falls during the first three components of the record, but is effective during the fourth, the amplitude of the second response being proportional to the amplitude of the 4th wave at the instant at which it occurs. It then decreases, sometimes to obliteration, when the stimulus follows the 4th wave, etc. (Bartley, 1936). The response to the second stimulus, when it occurs, contains all the elements exhibited by the first. After mild strychninization, as the late components decrease in amplitude, facilitation, even of the first wave of the second response, decreases, even though the

first component of the first response is several times its normal amplitude. A second response is finally effective only after a longer interval than normally, which is still more prolonged as the drug takes greater effect. The second response when it occurs is all or none and, like the first, consists only of the first diphasic component.

It thus appears that strychnine blocks the late component before it does the earlier. But even when it increases the amplitude of the early component, it tends to block this also, first increasing the latency of a first response and the least interval between effective stimuli, and finally rendering the cortex wholly inexcitable via the optic nerve. One of the most puzzling features of this situation is that the cortex may be difficult to activate, responding only to maximal stimuli, and then not to every one, at a stage when a *repetitive* response occurs when one occurs at all. The increase of amplitude and the repetitive character of the response are consistent, but the occurrence of excitation in this sense, and of blocking, at the same stage and with respect to the same elements of the response, can be given no present explanation.

We surmise that the increase of threshold, in this case connoting an increase in the number of optic nerve fibers which must be activated to cause a cortical response, may in part be assigned to a lack of the facilitation which normally occurs due to the spontaneous activity (Bishop and O'Leary, 1936) and which seems to occur at the level of the thalamus. That is, the depression of the spontaneous activity of the facilitating mechanism, as indicated by decrease of the late component of the response to stimulation as well as of the spontaneous rhythm, results in an increase of threshold for even the diphasic first component. The repetitive effect when the impulse has become effective at all must be a function of strychnine intoxication at the cortical level. At a more severe stage of intoxication, blocking occurs at the cortical level also, to both series of responses. This interpretation does not compromise the inference that the action of strychnine is differential, one process being increased in intensity, the other blocked without such an increase.

We may recognize, under this differentiation by strychnine, not only two events, but two parallel series of events corresponding presumably to facilitation, and to visual function respectively, as expressed in cortical potentials, from the very initiation of the cortical response to a nerve stimulus. It was previously observed (Bartley and Bishop, 1933) that in the normal cortex, with one lead on the surface and the other below it, the record of the initial elevation of the response was composite, consisting of a low monophasic wave and a high diphasic one, separable usually only near threshold stimulation. We now find that the monophasic component is recorded most strongly from leads near the middle third of the cortex. This potential has a lower threshold than the diphasic wave that obscures it when the stimulus is above threshold. The 3rd and 4th components

of the record follow this monophasic potential even below the threshold of the diphasic wave. Strychnine depresses the 3rd and 4th components together, and while the diphasic wave is so increased that the accompanying monophasic potential could not perhaps be detected if it occurred, it certainly is not increased similarly to the other, and is probably suppressed. We then have in one sequence three components: a small surface-positive initial wave, a larger and longer lasting surface-negative elevation following it (3rd component above), and the still later and larger surface-positive (4th) component. These are easily suppressed by strychnine, are associated with the spontaneous 5 per second rhythm, and are correlated with a cycle of cortical facilitation. A second sequence, comprising the two phases of the diphasic component, is increased in amplitude by strychnine, accounts for the spastic or tetanic strychnine "convulsions," and shows a behavior in general reminiscent of the action of strychnine on the spinal reflexes in its augmentation of intensity, all-or-none character, and increased latency. This latter sequence we take to be the one immediately concerned with the visual impulse.

Finally, this diphasic response itself can be further differentiated, into at least two parts corresponding to the two phases of the record, with their centers of activity in different strata of the cortex. It has been noted above that in the upper strata the first surface-positive phase predominates, in the middle both phases are about equally intense, and in the lower strata the second phase predominates. We cannot think of any way by which these phenomena could be interpreted as a single event, or even as the summation of similar events in many units. It suggests rather the conduction of the mass impulse vertically through the cortex, the reversal of polarity indicating either a change in direction of propagation, or a shift in the region of most intense activity (greatest negativity). The latter is suggested by the observation that if both leads are placed rather deep in the cortex, that is, if only the lowest stratum lies between them, the record may be diphasic in the reverse direction, that is, first phase surface-negative. Further, if a cut is made in the cortex, not extending quite to the white matter, a lead from a needle in the middle stratum of the cortex to one in the cut may record two waves each indicating negativity at the uninjured electrode, at the same time that leads from this electrode to one above it record first relative negativity, then positivity of the same point. Further information is required for even tentative interpretation of such records, but they do not seem to indicate any simple diphasicity in the sense in which it is applied to peripheral nerve.

SUMMARY

Two series of potentials can be detected in the record of the response to stimulation, in the optic cortex of the rabbit.

One series consists of a slow triphasic sequence, first phase surface-

positive, which is depressed only by deep anesthesia, and which is not increased in amplitude or duration by strychnine. Severe strychninization depresses it to extinction. It is related in many ways to the alpha rhythm. The inference can be made that a stimulus sets up in the cortex one alpha cycle, or a decrementing train of them.

The second series comprises a diphasic sequence, first phase again surface-positive, the waves of which have about $\frac{1}{10}$ the duration of the former series, which are typically superposed on the first wave of that series, and which do not repeat after a single stimulus. Ether readily depresses this sequence, and strychnine increases it in amplitude. After severe doses of strychnine, applied locally to the cortical surface, this wave appears spontaneously, either singly or in repetitive series, and the response to stimulation is repetitive.

At this stage of strychninization, both the alpha rhythm and the slow sequence of the response to stimulation which corresponds to it, are materially decreased or suppressed entirely from the record. Strychnine therefore results in the replacement of one repetitive phenomenon, the alpha rhythm, by a second, the strychnine tetanus, which correspond to the two parts of the normal response to a single stimulus.

These two types of response appear to occupy separate cortical structures. First, in the response to stimulation, certain parts of them are normally coincident, but can be differentiated by threshold and by weak strychnine. Second, under progressive dosage by strychnine, the fast component is increased in amplitude when the slow is still unaffected, and the slow component is finally depressed when the fast is maximally excitable. There is no evidence of a transfer of potential from one form of wave to the other. Third, the two impulses involved, as represented by centers of relative negativity, have different physical pathways as well as different temporal dimensions in the cortex.

The diphasic sequence is inferred to connote the immediate functional response of vision, the triphasic sequence to represent the action of a facilitating or integrating mechanism, because of the different behaviors of the two circuits under experimental manipulation.

A situation similar in fundamentals but differing in details of temporal and spatial relations is being investigated in the sensori-motor cortex of the cat in this laboratory, and a simpler situation exists in the pyriform olfactory cortex of the rabbit, which appears not to give rise to the slow potentials.

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ACID INHIBITION AND THE CEPHALIC (PSYCHIC) PHASE OF GASTRIC SECRETION

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In previous publications (1, 2) it was shown that the presence of tenth normal hydrochloric acid in the stomach and upper intestine causes a definite inhibition of the intragastric chemical and intestinal phases of acid secretion. Inhibition of the intragastric chemical phase of secretion was shown to be due primarily to the presence of acid in the stomach, since inhibition is very marked in whole stomach pouches. The intestinal phase is not inhibited by acid in the stomach, the inhibitory effect apparently occurring when the acid leaves the stomach and enters the upper intestine. It was also shown that when the stimulus for acid secretion is very potent, inhibition may not occur; for example, the simultaneous combination of the stimuli for the intragastric chemical and intestinal phases of secretion usually results in a marked secretion of acid in spite of the fact that either stimulus alone may be well inhibited. Histamine secretion is not inhibited by acid in the stomach and upper intestine (3), presumably because of the great potency of this type of stimulation.

In the present paper we wish to present the results of studies on the effect of acid in the stomach and upper intestine on the cephalic (psychic) phase of acid secretion.

METHODS. Five normal dogs were studied. Tenth normal hydrochloric acid containing 15 mgm. of phenol red per liter was placed in the stomach and the animals teased with fresh, finely ground beef liver. The animals were always fasted 24 hours or longer before the experiment, and hunger was usually evident. In a few experiments they were only allowed to see and smell the liver but in most experiments taste was also employed by allowing them to lick a spoon which had been dipped in the liver and wiped clean with the finger. The taste stimulus was applied intermittently for from 3 to 5 minutes at the beginning, middle and end of each half-hour period and between these the liver was placed very close to the animal so that sight and smell were active. The liver was always fed at the end of the experiment. In some animals marked interest in the liver was maintained throughout the two hour period, while in others there was evident

loss of interest after the second or third half-hour period, and in a general way these differences in behavior were reflected in the results.

Both the fractional and "Block" methods of analysis were used, gastric samples being removed every half hour for two hours. Details of the methods and calculations have been described in previous papers and will not be repeated.

RESULTS. *A. Tenth normal hydrochloric acid in the stomach with no stimulation.* When N/10 HCl was placed in the unstimulated stomach, the usual result was an acid deficit in the gastric samples due to the fact that no acid was secreted and some of the acid in the test meal was neutralized by the non-acid fluids secreted by the stomach and regurgitated from the duodenum. This is illustrated in the first three experiments in the upper half of the table. Occasionally secretion occurred as illustrated in the fourth experiment. The probable cause of this secretion will be referred to later.

B. The effect of tenth normal acid on the psychic phase of secretion. Twenty-seven experiments were performed on five dogs, all of which are shown in the figure and table.

Reference to the figure shows that a fairly high rate of secretion occurred in the majority of experiments. The secretion rate was usually highest in the early part of the experiment, frequently dropping rapidly toward the end of the two hour period; the sharp decline was often definitely associated with loss of interest on the part of the animal. The response varied considerably in the different animals, being greatest in dogs 1, 3 and 5, and definitely less marked in dogs 2 and 4. In the two latter animals, acid secretion was often completely inhibited and small acid deficits were obtained. In dog 4 the low response was associated with an evident lack of interest in the liver; dog 2, however, showed great interest in the liver, which was in striking contrast to the low response.

In dog 1 several experiments are shown in which sight and smell were the only stimuli used during the first and second half-hour periods and as seen in the figure the response was quite definite. In other dogs a much smaller response was obtained.

In dog 3 nausea occurred on several occasions and was associated with a very low response, thus illustrating that the same nervous pathway cannot be occupied simultaneously by two antagonistic reflexes, the effect of nausea predominating.

In dogs 2 and 4 when no acid was secreted, the acid deficits were much less than usually occurs when acid is placed in the unstimulated stomach.

It is interesting to note that when the psychic secretion of acid was high, the amount of non-acid fluid was also very high. In dog 1, for example, the non-acid fluid, and as a result the total fluid, was greater in amount in the psychic phase experiments than it was in experiments on the intestinal or intragastric chemical phases of secretion. This increase in non-acid fluid

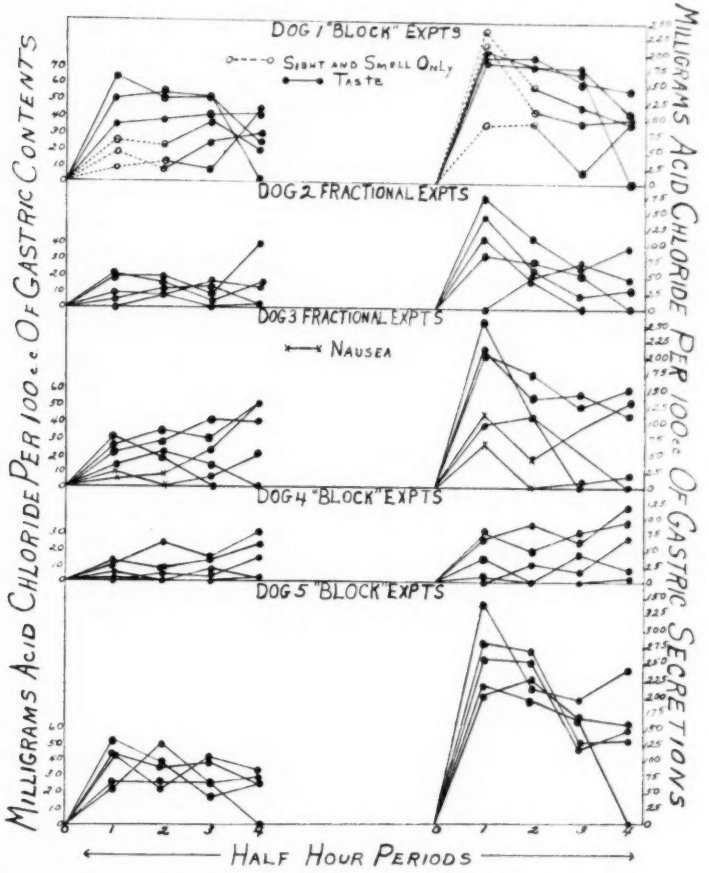

Fig. 1. Experiments on five normal dogs in which N/10 hydrochloric acid was placed in the stomach and psychic stimulation given by allowing the animal to see and smell ground liver or to lick a spoon dipped in the liver and wiped clean with the finger.

On the left side of the figure the results are expressed as milligrams of secreted acid chloride per 100 cc. of gastric contents and on the right side per 100 cc. of total secretions entering the stomach.

The difference between sight and smell only and taste is shown in dog 1.

The effect of nausea is shown in dog 3.

was not due solely to swallowed saliva since both the acid and non-acid secretions in response to psychic stimulation were abolished by thoracic vagotomy. This suggests that vagal activity may cause a secretion not only of acid, but also of non-acid fluid as demonstrated by Vineberg (4).

TABLE 1

Experiments on dog 1

ACID CHLORIDE ± THE VALUE CORRECTED FOR DILUTION		TOTAL FLUID	ACID FLUID	NON-ACID FLUID	TIME	REMARKS	CONDITIONS
Per 100 cc. of gastric contents	Per 100 cc. of secretion entering stomach						
mgm.	*mgm.*	*cc.*	*cc.*	*cc.*			
−5	−166	3	0	3	½		
−10	−200	5	0	5	½		
−10	−71	14	0	14	½		
−21	−150	14	0	14	½		
−3	−150	2	0	2	½		N/10 HCl test meal. No stimulation. "Block" experiments
+1	+33	3	0.2	2.8	½		
−10	−143	7	0	7	½		
−23	−256	9	0	9	½		
+2	+67	3	0.33	2.67	½		
−4	−100	4	0	4	½		
−1	−17	6	0	6	½		
−10	−143	7	0	7	½		
+31	+345	9	5.2	3.8	½		
+18	+164	11	3.0	8.0	½		
+9	+60	15	1.5	13.5	½		
+6	+35	17	1.0	16.0	½		
+51	+204	25	8.5	16.5	½	Taste in all	N/10 HCl test meal. Psychic stimulation. "Block" experiments
+53	+177	30	8.8	21.2	½		
+52	+168	31	8.7	22.3	½		
−25	−500	5	0.0	5.0	½		
+63	+185	34	10.5	23.5	½	Taste in all	
+51	+176	29	8.5	20.5	½		
+51	+176	29	8.5	20.5	½		
+25	+104	24	4.2	19.8	½		
+26	+236	11	4.3	6.7	½	Sight and smell only	
+21	+150	14	3.5	10.5	½	Sight and smell only	
+37	+123	30	6.2	23.8	½	Taste	
+20	+91	22	3.3	18.7	½	Taste	
+36	+189	19	6.0	13.0	½	Taste in all	
+38	+190	20	6.3	13.7	½		
+40	+160	25	6.7	18.3	½		
+43	+139	31	7.2	23.8	½		
+19	+211	9	3.2	5.8	½	Sight and smell only	
+8	+114	7	1.3	5.7	½	Sight and smell only	
+25	+96	26	4.2	21.8	½	Taste	
+29	+100	29	4.8	24.2	½	Taste	

Upper half. Experiments in which N/10 hydrochloric acid was placed in the unstimulated stomach. The high rate of secretion in the last experiment is possibly cephalic in origin.

Lower half. Experiments in which N/10 hydrochloric acid was placed in the stomach and psychic stimulation applied as explained in the text.

DISCUSSION. The above experiments show quite clearly that the psychic phase of acid secretion is able, in the majority of instances, to break through the inhibitory effect of acid in the stomach and duodenum and give rise to a fairly high rate of secretion. This shows that the intensity of the psychic stimulation may be very great, approximating the intensity of the simultaneously combined intragastric chemical and intestinal phases which are also able to break through the inhibitory effect of acid (2). Thus, the secretory energy of the psychic phase may often exceed that of either of the other two phases of secretion. This suggests that the cephalic phase may at times be responsible for a degree of hypersecretion and hyperacidity unattainable by either of the other two phases alone.

When tenth normal hydrochloric acid is placed in the stomach and no stimulation given, the usual result is that no acid is secreted and an acid deficit occurs (first 3 expts. upper half of table). Occasionally, however, a fairly high rate of acid secretion may occur (expt. 4, upper half of table). It appears quite probable that the secretion is due to cephalic stimulation. In the fasting human subject this could possibly be used as a test to determine whether or not a high resting secretion was due to a continuous cephalic phase.

While this work was in progress, a paper appeared by Day and Webster (5) in which they showed that the introduction of 0.25 per cent hydrochloric acid into the duodenum would decrease the *amount* of gastric secretion obtained by sham feeding two dogs with esophagotomy, gastric and duodenal fistulae and the stomach disconnected from the duodenum.

SUMMARY

1. The cephalic (psychic) phase of gastric secretion is usually able to break through the inhibitory effect produced by the presence of acid in the stomach and duodenum and cause a fairly high rate of acid secretion. This indicates that the intensity of the psychic stimulus often approximates that of the simultaneously combined intragastric chemical and intestinal phases which can likewise overcome the inhibitory effect of acid.

2. When tenth normal hydrochloric acid is placed in the empty unstimulated stomach, secretion of acid usually does not occur. When acid secretion does occur, it is probably the result of cephalic stimulation.

3. Since the secretory energy of the psychic phase may exceed that of the other two phases, it is possible for it to cause a degree of hyperacidity unattainable by either of the other two phases alone.

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STUDIES ON THE DYNAMICS OF THE PULMONARY CIRCULATION

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Examination of the reviews by Wiggers (1921), Hess (1930), Daly (1933), Hochrein (1935) and Wiggers (1937) reveals the fact that there has been no dearth of excellent and valuable studies upon the pulmonary circulation. But for the most part the studies have been made under highly artificial conditions or on abnormal animals. This depends largely upon the relative inaccessibility of the pulmonary vessels. Often the chest must be opened widely and artificial respiration maintained for the duration of the experiment, especially when adequate recording devices such as the Wiggers manometer are used. Abnormal intrathoracic pressures, faulty aeration of the blood and interference with the pulmonary circulation result. Often the experiments are carried out on animals in a state of shock caused by extensive hemorrhage and trauma. The studies have been largely analytical, dealing with isolated variables and with what can happen under artificial conditions. Such studies are valuable, but sometimes fail to give a true picture of what actually does happen under more nearly normal conditions in animals breathing naturally with an unimpaired circulation.

The development of an adequate pressure manometer of the "hypodermic" variety by Hamilton, Brewer and Brotman (1934) suggested a reinvestigation of the dynamics of the pulmonary circuit under more nearly normal conditions. With this technique one can record adequate curves from both large and small pulmonary vessels in lightly anesthetized animals without permanent pneumothorax or artificial respiration. The present studies deal with 1, pulse contours and pressure gradients in the pulmonary circuit, with and without pneumothorax; 2, velocity of the pulmonary pulse wave; 3, respiratory modifications in pulmonary arterial pressure; 4, the effects of increased resistance in the systemic circuit upon the pulmonary system; 5, the effects of peripheral vagus nerve stimulation and acetylcholine injection, and 6, the effects of asphyxia.

EXPERIMENTAL PROCEDURE. In these studies a temporary pneumo-

thorax was made on lightly etherized dogs by incising in the fourth or fifth interspace. The incision, about four inches long, could be made with very little hemorrhage. Intratracheal insufflation of air (and ether vapor) maintained the animal for the duration of the pneumothorax. Retraction of the ribs above and below the incision margins provided ample exposure of one lung and its vessels. Hamilton hypodermic manometers of appropriate sensitivity were equipped with glass cannulae which were inserted and tied into various vessels of the pulmonary circuit. This done, it was usually possible to close the pneumothorax opening about the leaden tubes of the manometers with several rows of sutures tightly enough to dispense with the artificial respiration, permitting the animal to breathe normally. A small cannula inserted into the chest cavity through a stab wound facilitated inflation of the lungs after closure of the pneumothorax opening, and also made possible the subsequent removal at intervals, of any air which might slowly enter the thorax through an imperfectly closed incision or from the bronchial system through small tears made incidental to exposure and cannulation of smaller pulmonary vessels embedded in the lung substance. By this technique normal intrathoracic pressure values and normal pressure fluctuations were maintained during many of the experimental procedures.

Manometers were also connected with vessels of the systemic circulation—with the carotid artery by means of a cannula or the thoracic aorta by means of a carotid sound. Shock was infrequent as shown by the high systemic arterial pressures obtained.

Results and discussion. 1. *Pressure pulses in the pulmonary circuit.* A typical record of the central pulmonary arterial and venous pulses in a closed-chested dog is shown in figure 1 A. Figure 1 B shows these same pressure curves along with the aortic pulse taken from a dog with a pneumothorax and a hypodynamic heart during administration of artificial respiration.

The contour of the pulmonary venous pulse is essentially the same in dogs breathing normally (1 A) or with pneumothorax (1 B), resembling pressure pulses reported previously in the auricles (Wiggers, 1928). As might be expected, the waves are seen more clearly when the heart is beating vigorously. Figure 1 A shows typical A, C and V waves, with the C wave—presumably caused by an arterial impact—coinciding with the rise of the arterial pressure as shown by the vertical alignment mark. In figure 1 A there is also a suggestion of an H wave. Figure 1 B clearly shows the notch of the semilunar value closure on the rising V wave.

The central pulmonary arterial pulse is smooth and free from superimposed oscillations, as in the majority of curves published by Katz (1925), Katz and Wiggers (1927) and Wiggers (1928). Only when the heart is hypodynamic (fig. 1 B) is there an anacrotic wave such as has

been reproduced in many current texts from an early curve published by Wiggers (1914).

Figures 1 C and D show a central pulmonary arterial curve taken simultaneously with the carotid arterial curve before and after intravenous injection of 1 mgm. epinephrine. The striking differences in contours of the carotid pulses under these two conditions are almost entirely absent from the pulmonary arterial pulses. Katz and Siegel (1929) and Katz, Ralli and Cheer (1928) have previously shown that marked changes in

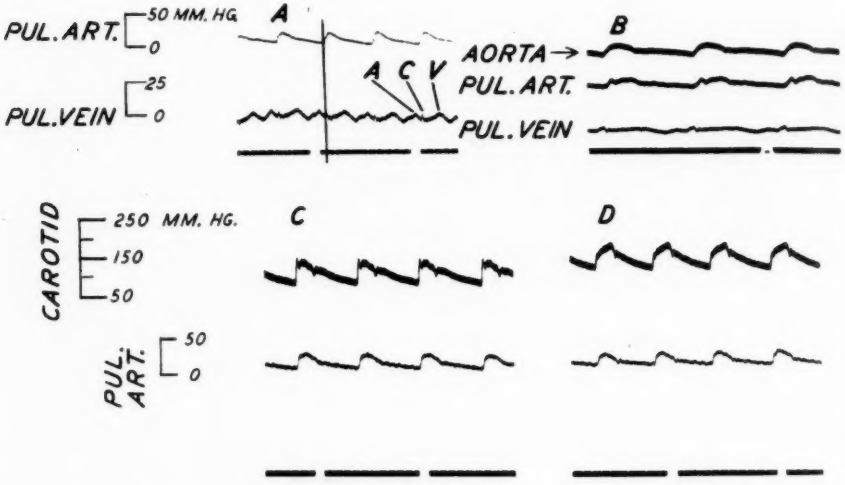

Fig. 1. Pulse curves in pulmonary vessels. *A*, central pulmonary arterial and venous pulses—normal respiration. *B*, same, plus aortic pulse under hypodynamic conditions. *C*, carotid and pulmonary arterial pulses. *D*, same after intravenous injection of 1 mgm. epinephrine. Tracings *B*, *C* and *D* were taken with the chest open. Time = 1 second.

the systemic arterial pressure pulse are not accompanied by changes in the pulmonary arterial pulse.

Figure 2 shows a series of central and peripheral pulmonary arterial pulses taken simultaneously with the aortic pressure curve. In A, B and C the chest was closed and the animal breathing normally. Tracing D was taken with the lungs collapsed by pneumothorax. Comparison of the central and peripheral pulmonary arterial pulse contours reveals the same changes as are found in the transformation of the systemic pulse: *a*, the anacrotus becomes steeper; *b*, a primary wave appears at the summit or is exaggerated; *c*, a secondary wave appears near the end of the

summit; *d*, the summit changes from a horizontal or ascending curve to a descent; *e*, a dicrotic dip followed by a dicrotic wave appears.

Apparently the same mechanisms as have been shown to operate in the transformation of the central to the peripheral pulse in the systemic circuit (Katz and Feil, 1925; Frank, 1926 and Wiggers, 1928) are also involved in the pulmonary circuit. A significant difference is that the transformations occur over a much shorter linear distance in the pulmonary circuit. In our experiments the cannulae in central and peripheral arteries were separated by a distance of only 12 cm. The transformation in the

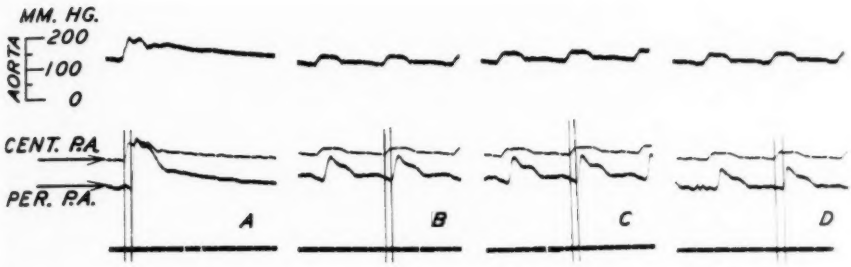

Fig. 2. Rate of transmission of the pulmonary arterial pulse wave. The aortic pulse (upper) is shown along with pulses from the pulmonary artery centrally (middle) and peripherally (lower). Time = 1/12 seconds.

Rate of transmission of the pulmonary arterial pulse wave

TRACING	PRESSURE IN CENTRAL PULMONARY ARTERY		CONDITION OF CHEST	DISTANCE BETWEEN CANNULAE	TRANSMISSION TIME	APPARENT LINEAR VELOCITY
	Diastolic	Systolic				
	mm. Hg	*mm.* Hg		*cm.*	*sec.*	*M./sec.*
A	6	40	Closed	12	0.033	3.6
B	15	30	Closed	12	0.032	3.7
C	15	30	Closed	12	0.030	4.1
D	10	25	Pneumothorax	7	0.041	1.7

character of the pulse seems therefore to depend but little upon the linear distance traveled, but apparently rather upon the amount of branching of the vascular tree. Absolute vessel calibre is not an important factor since central pulmonary vessels of a size comparable to the carotid artery have pulse contours very similar to those of the larger central pulmonary arteries.

2. *Pulmonary pulse velocity.* Figure 2 shows also that the onset of the pulse wave in the central artery precedes that of the peripheral artery, as we should expect. From this delay (indicated by vertical lines in the figure) it was possible to compute the rate of transmission of the pul-

monary pulse since the distance between the cannulae was known. The significant figures upon which the computations are based are given in the legend beneath figure 2. The transmission rate is of course much less than that normally present in the carotid artery (Bazett and Dreyer, 1922; Wiggers, 1923; Hallock, 1934), as would be expected from the higher pressure in the latter vessel. The velocity in the pulmonary vessels is higher than that calculated according to the formulae of Bramwell and Hill, 1922, for the peripheral systemic vessels at pressures equal to the pulmonary arterial pressures. It is interesting that the pulmonary pulse velocity is about the same as that of the aorta under normal conditions (Bazett and Dreyer, 1922; Wiggers, 1923) and much faster than that of the aorta at comparable pressures. This suggests that the aorta at these low pressures may be even more distensible than the large pulmonary vessels since pulse velocity is inversely related to distensibility. This rapid pulse velocity in the pulmonary vessels is determined by such factors as peculiarities of wall structure, profuse branching and the short distance over which the transition from large to small vessels occurs. It is interesting that the aorta and pulmonary arteries are equally distensible at the physiological pressures existing in each.

When the lungs were collapsed (tracing *D*, fig. 2) the transmission time for the shorter distance (7 cm. vs. 12 cm.) was increased or remained unchanged. This would suggest that the stretching and narrowing of the pulmonary vessels by inflation of the lungs accelerates the pulse velocity so that a greater distance is travelled in a shorter time. Whether similar effects of linear stretching of vessels are produced in the systemic circuit needs further study.

It is admitted that we do not know the exact distance travelled by the pulse in passing from central to peripheral cannulae, since any tortuosities of the vessels would be added to the actual linear distances measured. We refer to our findings therefore as "apparent" linear velocities.

3. *Effect of normal respiratory movements.* Figure 3 shows an intrathoracic pressure curve recorded simultaneously with the aortic pressure and the pressure in a small pulmonary artery located about $1\frac{1}{4}$ inches from the lung margin. The usual fluctuations in the systemic pressure are seen also in the pulmonary arterial pressure which rises in expiration and falls in inspiration even in the absence of any appreciable respiratory cardiac arrhythmia. The fluctuations in the pulmonary arterial pressure do not exceed the simultaneous changes in intrathoracic pressure and the two pressure curves run very nearly parallel. Thus it is not necessary to postulate changes in flow nor to speculate upon their delay in the lesser circulation in order to account for the changes in pressure (Visscher, Rupp and Scott, 1924). The thoracic pressure changes are probably the direct mechanical causes of the pulmonary arterial pressure changes.

Respiratory changes in flow, capacity and resistance occur under certain circumstances (Daly, 1930; Matthes and Hochrein, 1932; and many earlier workers) but when the venous pressure is normal and the heart is adequately filled, it is doubtful whether there are important respiratory changes in venous flow (Henderson and Barringer, 1913). In view of the ability of the pulmonary system to accept large changes in flow with negligible alterations in pressure it may well be doubted that respiratory fluctuations in systemic venous flow have much bearing upon respiratory variations in pulmonary arterial pressure. On the other hand, it is self-evident that intrathoracic pressure can directly and mechanically impress itself upon pulmonary arterial pressure (Wiggers, 1937). This applies as well to systemic arterial pressure (see Hamilton, Woodbury and Harper, 1936), and in the experiment illustrated (fig. 3) seems to be the only factor involved.

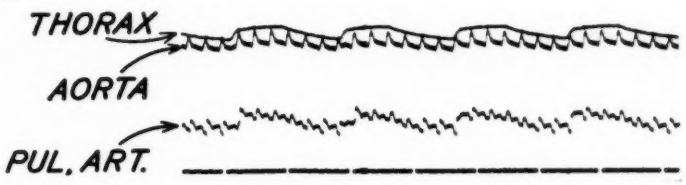

Fig. 3. Respiratory changes in intrathoracic (upper), aortic (middle) and pulmonary arterial (lower) pressures. Time = 1 second.

4. *Effects of increased peripheral resistance.* An increased systemic peripheral resistance produced by aortic compression in the heart-lung preparation causes marked changes in the pulmonary pressure (Anrep and Bulatao, 1925). In the "intact" animal with artificial respiration the pulmonary changes resulting from this procedure are slight (Katz, 1927; Katz and Wiggers, 1927; Katz and Siegel, 1929). The present findings on animals breathing normally confirm the latter observations as illustrated in figure 4 A. The aorta was occluded by tightening on a ligature placed about the lower thoracic aorta and passing through a sealed opening in the chest wall.

Aortic occlusion produced the usual marked rise in carotid pressure and sometimes a rise in jugular venous pressure. But despite the systemic arterial pressure rise of some 60 mm. Hg, the pulmonary arterial pressure rose very slowly by a total of only about 15 mm. Hg. The capacity of the pulmonary circuit to buffer extreme and sudden changes in systemic pressure is emphasized by these findings. This faculty of the lung vessels is even more strikingly illustrated by the results shown in figures 4 B and C. Here the intravenous injection of 1 mgm. of epinephrine induced a

rise in carotid pressure of over 100 mm. Hg in the course of a few seconds. In the pulmonary artery the pressure rose only about 30 mm. Hg,[1] and that only after a lag of one or two minutes. The right heart with its weaker musculature was thus spared the tremendous sudden strain to which the left heart was subjected.

Part of this sparing of the right ventricle is undoubtedly due to the ability of the left heart to adjust the force of its ejection to the arterial

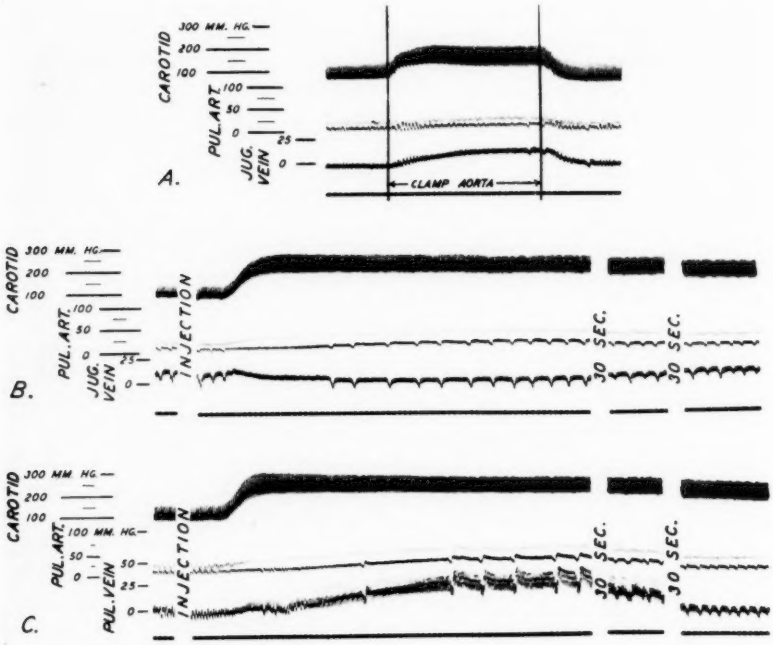

Fig. 4. A, the effect of partial occlusion of lower thoracic aorta upon the carotid (upper), pulmonary arterial (middle) and jugular venous (lower) blood pressures. B and C, effect of intravenous injection of 1 mgm. epinephrine upon the carotid (upper), pulmonary arterial (middle), jugular (B lower) and pulmonary venous (C lower) blood pressures. Dog atropinized, breathing normally. Time in seconds.

resistance through the operation of the Starling effect; part is due to the lowering of systemic venous pressure. But in large measure it must de-

[1] Our studies with epinephrine are at variance with those of Hochrein (1935) who found no increase in pulmonary pressure. Only in those experiments where the animals were in poor condition did we fail to obtain a rise in pressure. Apparently the results depend to some extent on the pulmonary blood content. This is lowered when the animals are in poor condition.

pend upon the great capacity of the pulmonary bed, enabling it to increase its blood content considerably without a great rise in pressure. Hamilton (1932), Hochrein and Matthes (1932, 1933) and Liebmeister (1934) present evidence for a marked increase in the quantity of blood in the lungs after epinephrine injection. Thus, despite a partial failure of the left heart, there is no great load placed immediately upon the right heart.

It might be suggested that the pulmonary arterial pressure rise after epinephrine injection is caused by pulmonary vasoconstriction since a

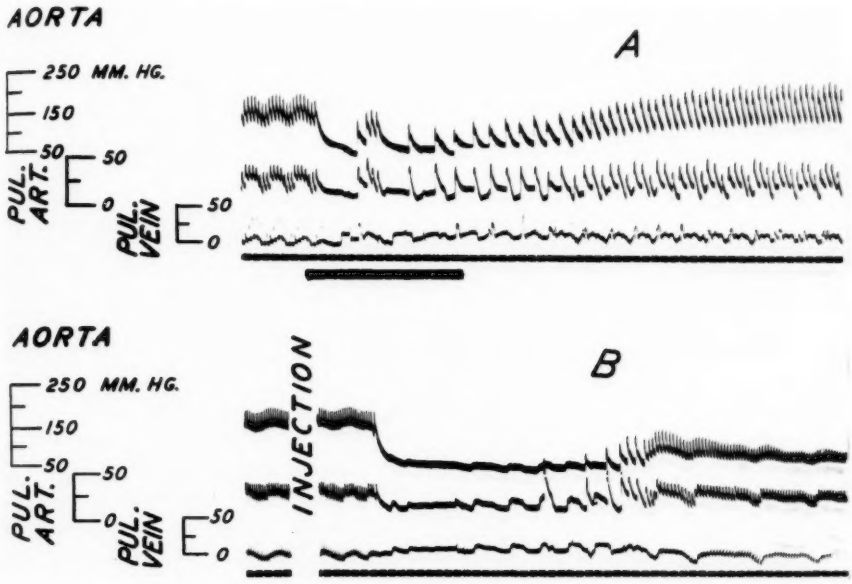

Fig. 5. Effect of stimulation of peripheral end of right vagus nerve (A) and intravenous injection of 40 mgm. acetylcholine (B) upon aortic (upper), pulmonary arterial (middle) and pulmonary venous (lower) blood pressures of a dog breathing normally. Time of stimulation shown by block below record A. Time in seconds.

number of experiments indicate that epinephrine can cause pulmonary vasoconstriction (see Daly, 1933). No one questions this explanation for the systemic arterial pressure rise, which, as seen in figure 4 B, is accompanied by a fall in jugular venous pressure. That is, there is an increased gradient of pressure from arteries to veins of the systemic system induced by the vasoconstriction. In the pulmonary circuit, however, the venous pressure rises parallel with the arterial. The pressure gradient remains about the same throughout, suggesting that pulmonary vasoconstriction had little if anything to do with the altered dynamics of the pulmonary

circuit. This point needs stressing in the light of the present tendency to emphasize the significance of drug and hormone effects upon blood vessels. We conclude that even large doses of epinephrine have but little effect directly upon the pulmonary circuit.

5. *Effect of peripheral vagus nerve stimulation and acetylcholine injection.* Stimulation of the peripheral end of the vagus nerve producing cardiac slowing lowers not only the systemic arterial pressure but the pulmonary arterial pressure as well (fig. 5 A) because of the reduced output of both left and right ventricles. Simultaneously, the pulmonary venous pressure rises. This would be expected because of the congestion of blood in the venous approaches to the hypo-active left heart. The effect seen would probably be related rather to this than to any vasomotor response of the pulmonary vessels (Daly, 1933) caused by the stimulation.

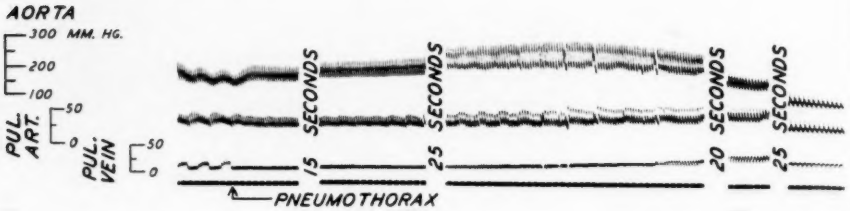

Fig. 6. Effect of asphyxia upon the aortic (upper), pulmonary arterial (middle) and pulmonary venous (lower) blood pressures. Time in seconds.

In figure 5 B is shown a similar effect from acetylcholine injection due again to a diminished cardiac output and congestion of blood in the pulmonary veins. In this instance the pulmonary venous pressure (15 mm. Hg) approaches very close to the pulmonary arterial pressure (18 mm. Hg). This small pressure gradient in the pulmonary circuit must be associated with an almost complete cessation of mass blood flow through the lungs. The sudden dropping off of the gradient from artery to vein probably leads to readjustments of the position of blood within the lung so as to give rise to backward currents in the capillaries of certain alveoli. Such reversed capillary flow has been directly observed by Hall (1925) and Macgregor (1933). However, we have seen no pressure gradient which might cause movement of blood from vein to artery through the lung as a whole.

6. *Effect of asphyxia.* Figure 6 shows results obtained in asphyxia induced by a pneumothorax without artificial respiration. The characteristic asphyxial rise in systemic arterial pressure is seen followed by a gradual fall as the left heart fails. The systemic pressure rise has but little immediate effect upon the pulmonary pressures. The initial small

rise is doubtless caused in the same manner as in aortic compression, and again it is probably the low elasticity coefficient of the pulmonary system that markedly limits the pressure rise. Pulmonary arterial and venous pressures rise most at about the time the systemic arterial pressure starts falling, i.e., when the left heart commences definitely to fail, being unable to eject its normal quota of blood. Finally, presumably when the right heart also fails, the pressure in the pulmonary vessels falls. Whether our finding that failure of the left heart precedes that of the right is the usual sequence in asphyxia we cannot say, but it serves to emphasize again the degree to which the right heart is physiologically protected against certain adverse conditions.

SUMMARY AND CONCLUSIONS

1. The pulse contours of peripheral and central arteries in the pulmonary circuit display differences much like those of the systemic circuit.

2. The pulmonary venous pulse rather closely resembles the systemic venous and auricular pulses.

3. The pulse velocity in the pulmonary arteries under normal conditions is nearly 4.0 meters per second, about the same as in the aorta at normal intra-aortic pressure.

4. Fluctuations in pulmonary arterial pressure with respiration are in the same direction as the variations in systemic arterial pressure, falling in inspiration, rising in expiration. The fact that these pressure changes parallel the alterations in intrathoracic pressure indicates that simple propagation of intrathoracic pressure fluctuations may play a prominent rôle in producing respiratory waves in pulmonary arterial pressure.

5. Pulmonary arterial and venous pressures rise together upon the injection of epinephrine. Whatever constrictor action the drugs may have upon the pulmonary arterioles is completely masked by the passive congestion of the lungs. This congestion results from the fact that the high systemic pressure has hindered the ejection of blood from the left ventricle.

6. Marked slowing of the heart, either from peripheral vagus nerve stimulation or acetylcholine injection produces a rise in pulmonary venous pressure simultaneously with a fall in the pulmonary and systemic arterial pressures. The lessening of the flow and hence of the pressure gradient between artery and vein, together with the elastic recoil of the pulmonary bed, seem sufficient to account for the phenomena observed.

7. Pulmonary arterial and venous pressures rise together in asphyxia, the rise being delayed until the heart commences to fail, as evidenced by the terminal fall in carotid pressure.

8. Marked sudden changes in systemic pressure have but little immediate effect upon the pulmonary arterial pressure, partly because of the

capacity of the pulmonary vessels to increase their blood content considerably with but little change in pressure.

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THE SPREAD OF THE IMPULSE IN THE MAMMALIAN VENTRICLE

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According to the widely accepted view of Lewis and his associates (1, 2), the normal impulse in the mammalian ventricle spreads rapidly through the subendocardial Purkinje network and then more slowly through the ordinary musculature of the ventricular walls to reach the epicardial surface with an asynchrony of several hundredths of a second between the first and last activated spots on the surface. De Boer (3), however, attributed these differences in conduction rate in part to the difference in temperature between the endocardial surface and the cooler exposed epicardial surface. Further, Lewis' results are not in accord with those of Clement (4) and Erfmann (5) who used the differential electrode, nor the more recent observations of H. Wiggers (6) who used an exploring electrode paired with one fixed on an injured spot so as to record monophasic curves. These investigators found little asynchrony (a matter of sigma) in the arrival of the impulse at various points on the heart's surface.

A second concept of impulse spread is that of Robb and her associates (7, 8) who have presented evidence to show that the impulse leaves the subendocardial Purkinje network and courses through the ventricular mass parallel to the superficial spiral muscle bundles and in a direction from apex to base. These investigators imply that the impulse travels with more facility along the muscle fibers than across them.

Recently, Cardwell and Abramson (9) and Abramson and Margolin (10) have demonstrated anatomically that the subendocardial Purkinje network joins with a myocardial plexus which spreads from endocardium to epicardium throughout practically all of the ventricular muscle mass. They found that these fibers for the most part ran perpendicular to the endocardium in the left ventricular wall and obliquely in the right ventricle. Transeptal Purkinje fibers were also described; i.e., fibers which arose from the subendocardial plexuses of both the right and left sides

[1] This work was done at the Michael Reese Hospital.

of the septum to penetrate the musculature, an observation previously made by Wahlin (11) in a single calf's heart. Some myocardial Purkinje fibers had been seen before by other investigators (see (9) for bibliography) but little importance had been attached to them. Very recently Robb (12) has admitted the functional significance of this network, but she still contends that the fibers follow along the muscle bundles and so conduct the impulse parallel to them.

It appeared to us that in order to test these alternate views, it would be desirable to perform the following experiments: *a*, to determine the time of arrival of the normal impulse at various points on the ventricular epicardium when the surface of the heart is kept moist and at body temperature; *b*, to determine the rate of spread of artificially induced impulses with respect to the direction of superficial muscle bundles; *c*, to determine whether or not cutting across the superficial muscle bundles will change the conduction time of artificially induced impulses spreading in a direction parallel to them; *d*, to determine the effect of cutting the right bundle branch near its origin on the conduction of an ectopic impulse from one ventricle to the other.

If Lewis' concept is correct, then *a*, his observations should be able to be confirmed on the heart kept warm and moist; *b*, the ventricular ectopic impulses should spread with equal facility in all directions, except that a delay might be found across the septum; *c*, cutting either along or across muscle bundles should have the same effect on the spread of ectopic impulses, and *d*, cutting the right bundle branch should significanlty delay the spread of an ectopic impulse from one ventricle to the other.

If Robb's concept is correct, then *a*, the ventricular ectopic impulses should spread with greater facility along the superficial muscle fibers than across them; *b*, cutting parallel to the muscle fibers should have little or no effect on the conduction of ectopic impulses across them, while transecting the muscle fibers should markedly delay conduction of impulses parallel to them, and *c*, the conduction time of the normal sinus impulse should become progressively greater as one follows along the superficial muscle bundles in a direction from apex to base.

On the basis of the anatomical findings of Abramson and his associates *a*, the normal sinus impulse should activate the ventricular surface with little asynchrony and no fixed pattern; *b*, the ventricular ectopic impulses should spread with equal rapidity in all directions on the ventricular surface, except for a delay across the septum (since myocardial Purkinje fibers are few or lacking in the anterior portion of the septum); *c*, cutting either along or across the superficial muscle bundles should have little or no effect on the conduction of ectopic impulses in either direction, and *d*, cutting the right bundle branch should not delay the spread of an ectopic impulse from one ventricle to the other.

PROCEDURE. The observations were made on a series of 12 dogs, average weight about 12 kgm., anesthetized with sodium barbital. The chest was opened and heart exposed; and to keep the surface temperature and humidity constant, the entire animal was enclosed in a large, glass-topped box, electrically heated and thermostatically controlled. Water-soaked sponges in the box kept the humidity high; several large holes in the sides were covered with sheet rubber, which was slit in order to allow easy access to the heart without lowering the temperature. The stellate ganglia were excised in order to slow the heart, since it was found that series of artificial premature beats could more readily be elicited under these conditions. In order to measure the time of arrival of the normal impulse, unipolar leads from various spots on the heart surface were re-

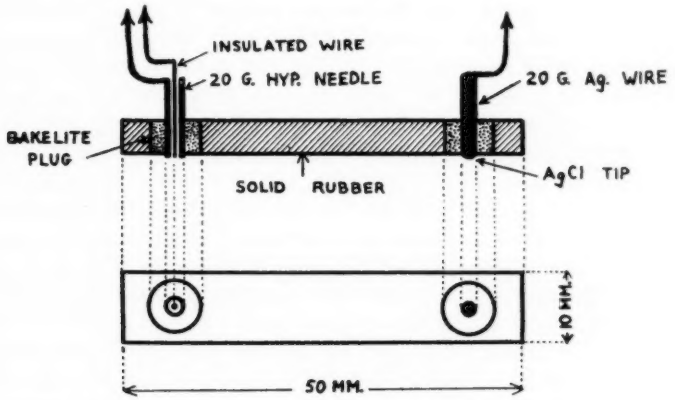


Fig. 1. Diagram of holder and electrodes used for initiating and recording ectopic ventricular impulses.

corded simultaneously with lead II. The unipolar lead consisted of a freely movable silver chloride-coated silver wire, the exploring electrode, paired with a similar type of electrode thrust under the skin of the groin. The exploring electrode was mounted in a bakelite plug $\frac{1}{4}$ inch in diameter with the tip of the wire barely projecting from the plug so that the electrode could be applied snugly to the heart without producing injury. The recording was done with either two Cambridge string galvanometers or a string galvanometer and a Victor machine.

In order to initiate and pick up the ectopic impulses, a special electrode holder (fig. 1) was used which was made of a strip of solid flexible rubber, in one end of which there was fixed an exploring electrode (similar to the one described above) paired with an indifferent electrode in the groin. The stimulating electrode, fixed in the other end of the holder, consisted

of a 20 gauge hypodermic needle with an insulated copper wire in the lumen. The tip of the needle, filed square, and the tip of the wire, thus formed concentric stimulating electrodes which allowed a minimal stimulus to be used with practically no spread of current. The flexible electrode holder could be applied to the surface of the heart to conform exactly with its curvature, so that the distance between the point of stimulation and the point of pick-up was always the same (30 mm.) along the heart's surface. The ectopic impulses were initiated by applying break shocks at a constant rate (slightly above the natural heart beat) from an inductorium driven by a Lewis interrupter. The exact moment of stimulation was indicated in the unipolar lead by a small sharp spike; this was produced by winding an auxiliary coil of 2 turns around the secondary of the inductorium and connecting it in series with the unipolar electrodes.

In all the unipolar leads the time of arrival of the impulse, whether normal or ectopic, was related to the apex of the initial wave of the electrogram. For the ectopic impulses, the conduction time was measured from the moment of stimulation (as demonstrated by the sharp spike) while for normal impulses the point of reference was the beginning of the QRS complex in the simultaneous lead II.

The tracings were all measured in millimeters with a Cambridge comparator (placed at our disposal by the Cambridge Instrument Co.). These readings were converted into fractions of a second by determining the distance in millimeters between the 0.04 second time lines on the record at the points measured. The magnification used was such that 0.001 second was equivalent to approximately 2 mm. The error of measurement was of the order of ± 0.0025 second. At least 3 cycles were measured for each determination and the average taken. In the case of the induced ectopic ventricular rhythm, the measurements were made only when a series of ectopic beats was obtained; those records containing occasional isolated complexes were not used. In making the measurements, the first two complexes of a series were disregarded since there was always the possibility that their time relationships might have varied depending upon where in the normal cycle the first effective stimulus had fallen. Curves showing injury currents were also disregarded.

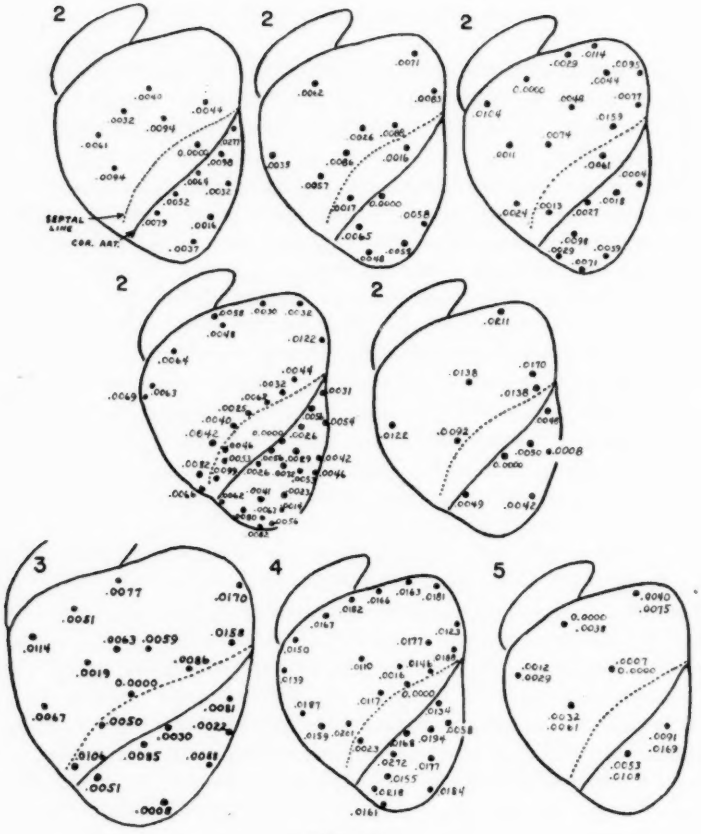
Discussion of results. 1. *The arrival of the normal excitation wave at the epicardial surface.* The results obtained in five experiments in which the animal was enclosed in the constant temperature box are given in figure 2. It will be seen that no definite pattern of spread exists under these conditions, except that in general the impulse first arrives on the surface near the middle of the anterior interventricular groove and late over the conus of the right ventricle. In all other regions the impulse arrives within, at most, 0.0138 second of its first appearance on the sur-

face.[2] These differences are only slightly outside the experimental error involved in the measurements. In all these experiments the heart's surface was kept moist and its temperature was approximately equal to body temperature.

In only one of three experiments in which the heart was exposed to room temperature were the results similar to those above (fig. 3), and in this case it was found that the surface temperature was practically equal to that of the body. In the other two experiments (figs. 4 and 5) where the surface temperature was lower than that in the animal's body, the results were more nearly like those reported by Lewis (2). In experiment 7 (fig. 5) comparisons were made of the time of impulse arrival at the same spots before and after enclosing the animal in the temperature-controlled box. In each instance there was a small but consistent increase in this figure when the heart surface was cooled, the delay being somewhat greater for sites on the left ventricle than for those on the right. It would seem, therefore, that the difference between the results of Lewis on the one hand and Erfmann, Clement and H. Wiggers on the other could be explained, partially at least, on the basis of 1, variations in surface temperature, as de Boer suggested, and 2, differences in the degree of surface drying. A sequential activation of the surface with respect to muscle bundles was not observed. In other words the results fail to support Robb's view that the impulse travels parallel to muscle bundles in a direction from apex to base. Further evidence against this contention was obtained in two experiments in which cuts were made across superficial muscle bundles and the effect upon the time of arrival of the normal impulse at sites between the cut and the base of the heart was noted. As will be seen (fig. 6), no delay was generally present following cuts (2–4 mm. in depth, 1.5 cm. in length) transecting superficial sino-spiral fibers in the right ventricle or superficial bulbo-spiral fibers in the left. The slight changes sometimes observed were no greater in those in-

[2] There is one exception, a spot near the base of the left ventricle on the anterior surface near the septum, which showed a lag of 0.0277 second in one experiment. This is out of line with our other data. Certain fine oscillations are present in this record and we are inclined to view it as an artefact such as H. Wiggers (6) refers to.

There is an apparent paradox in the fact that the greatest difference in time of arrival of the normal impulse on the surface of the heart is less than the duration of the QRS complex in indirect leads; in fact, it is less than the time from the beginning to the peak of the major deflection. This can be understood if one remembers that the QRS complex records the difference in time of onset not only on the surface, but also in the septum and other portions of the myocardium, which of course are not indicated in surface measurements. Furthermore, the QRS records not only difference in time of onset, but also difference in the time required for full activation to develop in the various spots.



Figs. 2–5

Fig. 2. Results of five experiments showing the time of arrival of the normal sinus impulse at various spots on the surface of the ventricles when the surface was kept moist and at body temperature. The figures indicate, in seconds, the time of arrival of the impulse with reference to the earliest activated spot on the surface measured. Top row are experiments 4, 8 and 10, bottom row, experiments 11 and 12.

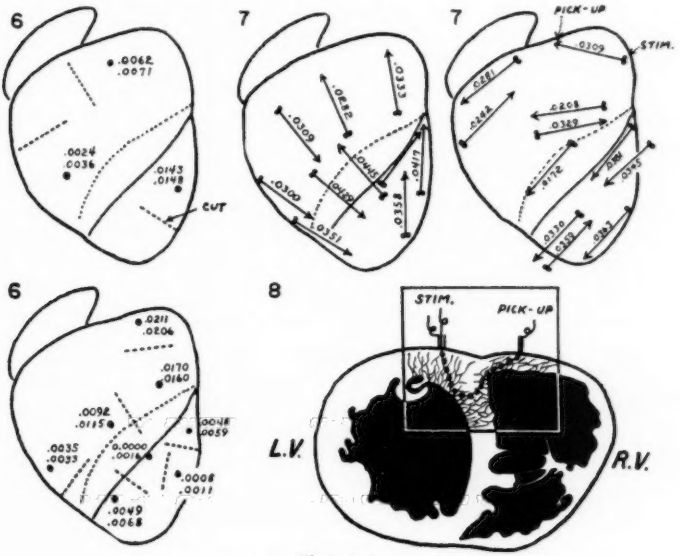
Fig. 3. Results of one experiment (expt. 6) showing the time of arrival of the normal sinus impulse when despite the exposure of the heart surface to room temperature its temperature still closely approximated that of the body. Heart surface temperature varied from 39° to 40.5°, while rectal temperature simultaneously rose from 39° to 41°.

Fig. 4. Results of a typical experiment (expt. 9) showing time of arrival of normal sinus impulse when exposure to room temperature lowered the heart surface temperature below that of the body. Surface temperature was 38.2° and rectal temperature was 39.6°.

Fig. 5. Results of a typical experiment (expt. 7) showing time of arrival of normal sinus impulse at same spots both with and without constant temperature box. Upper reading was made with heart surface at body temperature, lower reading with surface at cooler room temperature.

stances in which the site was basal to the cut than for those in which the cut was either nearer the base or parallel to the muscle fibers.

2. *The spread of the artificially induced impulse.* a. *In the normal heart.* The results obtained with the electrode holder previously described are shown in table 1 and figure 7. It will be seen that in each experiment and for the various positions no significant difference was found in the conduc-



Figs. 6–8

Fig. 6. Results of two experiments showing effect of myocardial cuts along and across superficial muscle bundles on time of arrival of normal sinus impulse. Upper reading before cut, lower reading after cut. Experiment 7 above, experiment 11 below.

Fig. 7. Results of a typical experiment (expt. 12) showing conduction time in various directions and regions of artificially induced ectopic impulse over a fixed distance on the epicardial surface.

Fig. 8. Diagram showing anastomoses of myocardial Purkinje fibers in the septum and the probable pathway taken by an ectopic impulse originating on the surface of one ventricle and passing across the septum to the surface of the other ventricle.

tion time of the impulse from the site of stimulation to the site of the recording electrode a fixed distance away, provided only that both were confined to one ventricle. No correlation was noted between conduction time and direction of muscle bundles. When the recording electrode was on the opposite ventricle to the stimulating one, a delay, sometimes of as much as 0.01 second, was apparent in all but a few instances. In

TABLE 1

Time taken by induced ventricular ectopic impulse to spread from stimulating to recording electrode 30 mm. away

EXPERIMENT NUMBER	RELATION OF LONG AXIS OF ELECTRODE HOLDER TO SUPERFICIAL MUSCLE FIBRES	ELECTRODE HOLDER ON RIGHT VENTRICLE			ELECTRODE HOLDER ON LEFT VENTRICLE			ELECTRODE HOLDER ACROSS INTERVENTRICULAR GROOVE ON BOTH VENTRICLES			ELECTRODE HOLDER ON INTERVENTRICULAR GROOVE PARALLEL TO LINE OF GROOVE		
		Number of sites	Average time	Average apparent rate of spread	Number of sites	Average time	Average apparent rate of spread	Number of sites	Average time	Average apparent rate of spread	Number of sites	Average time	Average apparent rate of spread
			sec.	*mm./sec.*		*sec.*	*mm./sec.*		*sec.*	*mm./sec.*		*sec.*	*mm./sec.*
1	Parallel							4	0 0385				
	Right angle	2	0 0320		2	0 0314							
	Angle												
	All sites	2	0 0320	937	2	0 0314	955	4	0 0385	778			
2	Parallel	8	0 0304		2	0 0331		7	0 0490				
	Right angle	2	0 0363								1	0 0307	
	Angle	4	0 0333										
	All sites	14	0 0321	937	2	0 0331	906	7	0 0490	612	1	0 0307	977
3	Parallel	11	0 0287					7	0 0408				
	Right angle	10	0 0271								1	0 0347	
	Angle	2	0 0259					1	0 0310				
	All sites	23	0 0281	1077				8	0 0383	780	1	0 0347	864
4	Parallel	4	0 0383		1	0 0445		4	0 0607				
	Right angle				3	0 0414					2	0 0434	
	Angle	1	0 0351										
	All sites	5	0 0377	795	4	0 0422	710	4	0 0607	493	2	0 0434	691
7	Parallel	3	0 0230		2	0 0399		1	0 0317				
	Right angle	2	0 0232										
	Angle												
	All sites	5	0 0231	1294	2	0 0399	751	1	0 0317	943			
8	Parallel	3	0 0382		2	0 0351		2	0 0328				
	Right angle	6	0 0332		3	0 0446							
	Angle	2	0 0302										
	All sites	11	0 0341	879	5	0 0408	733	2	0 0328	911			
9*	Parallel	4	0 0428		5	0 0493		6	0 0655				
	Right angle	9	0 0467		3	0 0509					1	0 0558	
	Angle	5	0 0484										
	All sites	18	0 0455	659*	8	0 0498	602*	6	0 0655	456*	1	0 0558	535*
10	Parallel	2	0 0323		4	0 0351		1	0 0414				
	Right angle	2	0 0314										
	Angle	1	0 0337		1	0 0391							
	All sites	5	0 0323	925	5	0 0359	832	1	0 0414	724			
11	Parallel	2	0 0323		2	0 0363							
	Right angle	3	0 0328										
	Angle				2	0 0368							
	All sites	5	0 0326	920	4	0 0366	819						
12	Parallel	9	0 0308		9	0 0347		6	0 0407				
	Right angle	5	0 0274								1	0 0172	
	Angle				4	0 0354							
	All sites	14	0 0295	1017	13	0 0350	857	6	0 0407	737	1	0 0172	1744

* In experiment 9 the control temperature moist box was not used. In all other experiments it was employed.

addition, the average time of spread on the left ventricle was longer in all but one experiment than that on the right ventricle. In individual instances it was found that reversing the position of the stimulating and recording electrodes occasionally caused noticeable differences in the time of impulse spread. Cooling the heart's surface by exposing it to room temperature also slowed the conduction rate.

The fact that the conduction time along and across muscle fibers limited to one ventricle was approximately the same while the impulse took much longer to travel the same distance across the septum from one ventricle to the other contradicts Robb's views. The latter finding also precludes the possibility that the ectopic impulse travels *only* over muscle fibers; for under such conditions, since there are continuous superficial muscle bands crossing the septum, the conduction time along these bands should have been the same as along those limited to any one ventricle. The fact that the conduction time for ectopic impulses on the left ventricle was in general somewhat longer than that on the right might be conceived to agree with the postulates of Lewis (1, 2), Drury and Mackenzie (13) and Dale and Drury (14). According to them the ectopic impulse arising on the epicardial surface travels to the endocardium via ventricular muscle and then over the subendocardial Purkinje tissue and back through muscle again to reach the pick-up electrode on the epicardium. If this is so, then a different rate of spread from that given by Lewis would have to be assumed for the impulse in its passage through ventricular muscle. Thus, the difference in thickness of the two ventricular walls is, on the average, 4 mm. (7 mm. for left less 3 mm. for right). An ectopic impulse in the left ventricle, then, would have to traverse 8 mm. more of muscle than one in the right, and assuming the rate given by Lewis of 400 mm./sec., the average conduction time on the former should be 0.02 second longer than that on the latter, rather than only 0.005 second which was actually found.

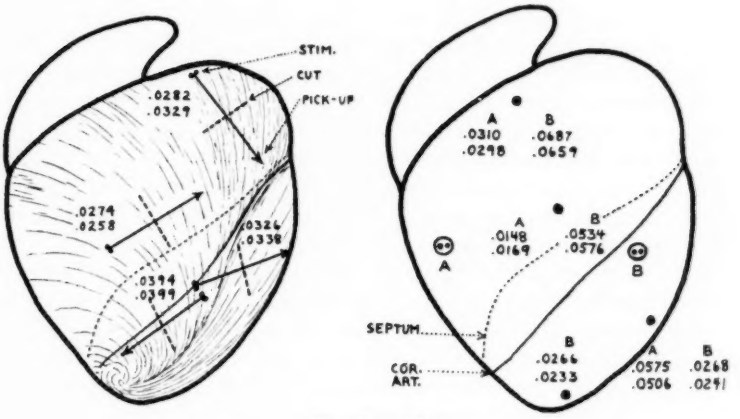
On the other hand, these results are in accord with the anatomical findings of Abramson et al. (9, 10). In view of the presence of a widespread Purkinje network in the muscle itself, it is not necessary to postulate that the impulse must enter the subendocardial Purkinje network in order to reach the epicardium in the shortest possible time, but rather that it spreads from its origin to the site of the pick-up electrode via these anastomosing Purkinje fibers present close to the epicardial surface. The differences in time consumed by the impulse in travelling the specific distance in the various locations on any one ventricle can be attributed merely to variations in the extent and complexity of the myocardial Purkinje network in these sites. Further, the somewhat smaller figures generally obtained on the epicardial surface of the right ventricle can be explained by the fact that the wall of this ventricle is rather thin in some parts; accordingly, the impulse may have entered the subendocardial

plexus, and because of the greater accumulation of fibers in this location, it may have reached the recording electrode by a more direct course (and consequently sooner) than in the case of the left ventricle, where it would have to extend over a comparatively sparser Purkinje system in the myocardium.

Further evidence for the above view is obtained when we consider that the only locations in which a delay in the spread of the ectopic impulse was almost invariably noted contained very few, if any, myocardial Purkinje fibers. For, according to anatomical studies (15), the Purkinje fibers which traverse the septum connecting one subendocardial network with the other, are present in the central part of the septum (in relation to the antero-posterior surfaces) rather than in those portions underlying the interventricular grooves. Accordingly the impulse travelling from the surface of one ventricle to that of the other, if it remained in the Purkinje system, must have followed a more tortuous path than one passing between two points on a single ventricle (fig. 8), thus resulting in an increase in the time of conduction. Another alternative is, of course, that the impulse spreads directly across the surface of the septum over muscle fibers intervening between the two ventricles, but if this is so then we must assume that the rate of spread over this shorter pathway is much slower than that over Purkinje tissue.

No attempt has been made to present actual rates of conduction over Purkinje fibers in the muscle, but merely average figures; for it is realized that the pathway taken by the artificially propagated impulse from its origin to the site of the pick-up electrode must have been tortuous and variable, and therefore longer than the measured epicardial distance between the two electrodes. Moreover, figures supposedly representing the actual rate of spread of the induced ectopic impulse, obtained with the above procedure, may be open to criticism on other grounds. First, there may have been a latent period between stimulation and the local response, although it is unlikely that this latency would have been of significant magnitude (Fulton, 16). Secondly, on the assumption that an impulse travelling antidromically is conducted more slowly than one going in the normal direction, there may have been a type of block either at junctions of Purkinje fibers with ordinary muscle (Rijlant, 17), or in the Purkinje tissue itself. The phenomenon of unidirectional conduction in the specialized tissue of the A-V node and common bundle is well known experimentally and clinically, and unidirectional conduction in heart muscle has been investigated by Schmitt and Erlanger (18) and Ashman and Hafkesbring (19). It is not unlikely that unidirectional facility of spread of the impulse was present, and our results would favor this conception. The fact that marked differences in conduction time sometimes occurred when the positions of the recording and stimulating electrodes were reversed also is in accord with this view.

b. *Following deep cuts in the heart.* To test Robb's hypothesis further, the effect upon the time of conduction of making a deep cut between the recording and stimulating electrodes was investigated. After taking a control record, the electrode holder was removed and a cut 2 to 4 mm. deep (the deeper ones being made in the left ventricle) and 15 mm. long was produced with a cautery knife, at right angles to the line joining the positions of the two electrodes. The electrode holder was replaced in



Figs. 9 and 10

Fig. 9. Results of a typical experiment (expt. 11) showing the effect of myocardial cuts on the conduction time of artificially induced ectopic impulses over a fixed epicardial distance. The cuts were always made at right angles to the line between stimulating and pickup electrodes and at various angles to the direction of muscle bundles. Upper readings before cut, lower readings after cut.

Fig. 10. Results of one experiment (expt. 2) showing effect of complete transection of right bundle branch (checked postmortem) on conduction time of artificially induced ectopic impulses from the same and opposite ventricle. A and B indicate sites of stimulation; at each pick-up spot the figures under A represent conduction time for impulse originating at A, and those under B represent conduction time for impulse originating at B. Upper figures before, lower figures after transecting the right bundle branch.

exactly the same position and premature ventricular contractions again elicited and the electrograms recorded. The results are shown in figure 9.

It will be noted that the changes are of the same order, regardless of whether the cut was across or parallel to the muscle bundles. In fact, no significant interference with conduction was noted following cuts anywhere in the myocardium, an observation previously reported by Lewis (2). These results, we feel, definitely oppose the view that the ectopic impulse travels parallel to muscle bundles. On the other hand, they are in accord with the concept of impulse spread over myocardial Purkinje fibers. For

with the presence of a widespread network in the muscle, destruction of a comparatively few fibers should not markedly delay the passage of the ectopic impulse from site of origin to recording electrode.

c. *Following transection of the right branch of the bundle of His.* The greater lag found in the spread of ectopic impulses from one ventricle to the other was explained above as due to the devious pathway taken by the impulse in passing through the septal myocardial Purkinje fibers. The classical concept, however, following the views of Lewis, was that the impulse arising in one ventricle had to pass up the bundle branch of that ventricle to the bifurcation of the bundle of His and then down the other branch to reach the opposite ventricle. In order to test these possibilities, two experiments were done; the results are shown in figure 10. A pair of fish hook electrodes was attached to the surface of each ventricle, and the heart was driven artificially from these sites in turn. The time of arrival of the induced impulse at various points on the same and opposite ventricle was determined before and after cutting the right bundle branch. In each instance it was found that the time of spread of the impulse to the opposite ventricle was definitely longer than to sites on the same ventricle. Sectioning the right bundle branch, however, while delaying the time of arrival of the sinus impulse to the right ventricle, had no significant effect on the time of spread of ectopic impulses from either the same or the opposite ventricle. This is in accord with the observations of Rothberger (20) and also with some of the results of Drury and Mackenzie (13); it is contrary to Lewis' findings (1), (2) and those of Dale and Drury (14). Drury and Mackenzie (13) believed that there was a race between 1, spread of the impulse up the bundle branch to the bifurcation of the bundle of His and down the other bundle branch, and 2, spread across muscle. Since only a limited number of sites were studied in the present work, the first possibility cannot entirely be exluded. However, on the basis of the above findings as well as in the light of the existence of myocardial Purkinje fibers traversing the interventricular septum, it would appear much more reasonable to assume that the impulse utilizes the latter pathway rather than the two postulated by Drury and Mackenzie.

SUMMARY

The subject of impulse spread in the ventricles was investigated in a series of 12 dogs. The surface of the heart was prevented from drying, and its temperature was maintained at body level. Under these conditions, it was found that the normal sinus impulse reached the epicardial surface of both ventricles almost simultaneously.

It was found further that no correlation could be made between the rate of propagation of an artificially induced impulse on the one hand and

the relationship of the points of stimulation and pick-up with respect to the direction of the superficial muscle bundles on the other. In other words, the impulse did not spread any more rapidly when the points were situated along the muscle bands than when they lay in a line across them. When the stimulating electrode was on one ventricle and the recording electrode on the other, a marked delay in the spread of the artificially induced impulse was noted, even when the two electrodes were placed in a line parallel to the superficial sino-spiral fibers which cross the anterior interventricular sulcus. By noting the effect of epicardial cuts on the rate of propagation of the artificially induced impulse, further evidence was collected against the hypothesis that the excitation wave spreads parallel to the superficial muscle bundles in a direction from apex to base.

The average time consumed in the spread of an artificially induced impulse over a constant epicardial distance on the right ventricle was found to be only slightly less than that on the left. In view of the marked difference in thickness of the outer walls of the two ventricles, this finding is opposed to the concept that an artificially induced impulse arising on the epicardial surface of either ventricle penetrates the muscle wall at a slow rate of speed, enters and spreads rapidly through the subendocardial Purkinje network, and then traverses muscle again to reach the epicardium.

By obtaining the times of arrival of artificially induced ectopic impulses at various spots before and after cutting the right branch of the bundle of His, evidence was found which indicated that the impulse originating on one ventricle might reach the opposite one through the myocardial Purkinje fibers in the septum, rather than over the bundle branch pathway via the bifurcation of the bundle of His.

Since the data could not for the most part be reconciled with the current concepts of impulse spread in the ventricles, an attempt was made to correlate the results with the anatomic findings of a widespread myocardial Purkinje network, which up to the present has not received physiological significance. On the basis of the results herein presented, it seems justifiable to assume that the impulse does not leave the subendocardial Purkinje network to spread out over muscle itself, but rather that it remains in the continuation of the network, i.e., in the above-mentioned myocardial Purkinje fibers, and, spreading out over this extensive plexus, excites the muscle in numerous places almost simultaneously.

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